

# **Collaborative meta-analysis finds no evidence of a strong interaction between stress and 5-HTTLPR genotype contributing to the development of depression**

Running title: **5-HTTLPR, stress, and depression collaboration**

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## Abstract:

The hypothesis that the S allele of the 5-HTTLPR serotonin transporter promoter region is associated with increased risk of depression, but only in individuals exposed to stressful situations, has generated much interest, research, and controversy since first proposed in 2003. Multiple meta-analyses combining results from heterogeneous analyses have not settled the issue. To determine the magnitude of the interaction and the conditions under which it might be observed, we performed *new* analyses on 31 datasets containing 38 802 European-ancestry subjects genotyped for 5-HTTLPR and assessed for depression and childhood maltreatment or other stressful life events, and meta-analyzed the results. Analyses targeted two stressors (narrow, broad) and two depression outcomes (current, lifetime). All groups that published on this topic prior to the initiation of our study and met the assessment and sample size criteria were invited to participate. Additional groups, identified by consortium members or self-identified in response to our protocol (published prior to the start of analysis<sup>1</sup>) with qualifying unpublished data were also invited to participate. A uniform data analysis script implementing the protocol was executed by each of the consortium members. Our findings do not support the interaction hypothesis. We found no subgroups or variable definitions for which an interaction between stress and 5-HTTLPR genotype was statistically significant. **In contrast, our findings for the main effects of life stressors (strong risk factor) and 5-HTTLPR genotype (no impact on risk) are strikingly consistent across our contributing studies, the original study reporting the interaction, and subsequent meta-analyses.** Our conclusion is that if an interaction exists in which the S allele of 5-HTTLPR increases risk of depression only in stressed individuals, then it is not broadly generalizable, but must be of modest effect size and only observable in limited situations.

## INTRODUCTION

Depression negatively impacts health more than any other chronic disease<sup>2</sup> and is a leading cause of total disease burden worldwide.<sup>3</sup> Both genetic and environmental factors influence depression;<sup>4</sup> research on the etiology of depression suggests substantial heritability of 40-50%.<sup>4-9</sup> Only recently have genome-wide association studies (GWAS) begun to identify and replicate specific loci associated with depression.<sup>10-12</sup> The findings from these studies suggest that (1) the effects of individual single nucleotide polymorphisms (SNPs) on major depressive disorder (MDD) are small in magnitude (requiring large sample sizes to detect), and (2) candidate genes generally do not show evidence of association in either GWAS or subsequent large-scale meta-analyses.<sup>13</sup> Gene-environment interactions (GxE) (e.g., genetic variants whose influence on depression risk is only seen under specific environmental exposures) are one mechanism that may contribute to the complexity of identifying genetic associations with depression.<sup>14, 15</sup>

A high profile report of a GxE effect on the development of depression involves an interaction between stressful life events and a functional, repeat length polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene (*SLC6A4*) on chromosome 17.<sup>16</sup> *SLC6A4* encodes an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons. The short (S) allele of 5-HTTLPR is associated with less transcription of the serotonin transporter compared to the long (L) allele.<sup>17, 18</sup> The report found that carriers of either one or two copies of the S allele of 5-HTTLPR were more likely to develop major depressive disorder, increased depressive symptoms, and suicidality in response to childhood maltreatment or other stressful life events than were individuals homozygous for the L allele. Furthermore, there was evidence of a dose-response relationship, with risk of depression higher amongst those with two copies of the S allele compared to individuals with only one copy in the presence of stress. This GxE interaction report has had considerable influence on the field; it has been cited over 4000 times and over one hundred publications have investigated the combined impact of 5-HTTLPR variation and stress on risk for depression.

However, controversy over the robustness of this GxE interaction continues. Although it is likely that GxE interactions play an important role in disease, gene-by-environment studies are challenged by the fact that statistical power to detect interactions is typically less than for main effects.<sup>19</sup> Furthermore, many candidate gene main-effect association reports appear to be false positives.<sup>20, 21</sup> As Duncan and Keller<sup>22</sup> illustrate, this indicates a need for caution regarding similar gene-by-environment hypotheses. Several meta-analyses have examined the 5-HTTLPR-by-stress hypothesis, some providing support for the interaction and others finding no evidence for it,<sup>23-26</sup> with various reasons proposed for the differences.<sup>22, 25, 27-29</sup> Munafò et al.<sup>24</sup> performed a literature-based meta-analysis, finding that only 5 of the previously published studies (N = 2 999) used phenotypes and statistical models suitably comparable to the original study to be included in the meta-analysis. This meta-analysis did not support replication of the original finding. Risch et al.<sup>23</sup> obtained individual level data from 10 previously published studies (N= 14 250) that met inclusion criteria and analyzed the data using a common model based on number of stressful life events. This re-analysis found no evidence for either a main effect or interaction effect of 5-HTTLPR on depression. Karg et al.<sup>25</sup> (56 studies, N= 40 749) and Sharpley et al.<sup>26</sup> (81 studies, N= 54 996) both performed literature-based meta-analyses and reported strong evidence for the interaction. Karg et al. and Sharpley et al. criticized the previous analyses of Munafò et al. and Risch et al. for being too restrictive in their inclusion of studies. The approaches of Karg et al. and Sharpley et al., in turn, have been criticized for combining p-values too broadly by allowing studies with an opposite direction of effect to supply supportive evidence, by including results from studies with incompatible statistical and genetic models, and by including outcomes other than depression.<sup>22</sup> One key issue contributing to disputes over the appropriateness of the prior reports and meta-analyses is the heterogeneity of the studies.<sup>22</sup> Heterogeneity pervades many key factors in the prior analyses, including measurements of depression and stress, genetic ancestry, and statistical models.

The primary objective of the current study was to increase understanding of the role 5-HTTLPR might play as a moderator of the response to stress as it impacts depression. To address the complexities of this topic, we performed a collaborative meta-analysis of data available from the participating studies, both published and unpublished, using consistent *de novo* analyses and variables determined *a priori*

as described in the pre-registered protocol.<sup>1</sup> Our collaborative meta-analysis strategy, wherein the consortium worked to harmonize phenotypes across studies, to prioritize specific analyses *a priori*, and to apply identical *de novo* statistical analyses across all participating studies, provided a balance between maximizing sample size while minimizing heterogeneity. With this approach and the large number of contributing samples, we are well positioned to clarify the relationship between 5-HTTLPR, stress, and depression.

## **METHODS**

### **Coordinated meta-analysis process**

#### **I. Recruitment of studies**

Our goal was to include data from as many pertinent studies as possible. However, analyses based on a small number of samples can be statistically unstable, a problem that is exacerbated in models involving multiple covariates and an interaction term. For these reasons, we required participating studies to have genotyped at least 300 individuals for 5-HTTLPR and to have assessed depression and stress for inclusion. Our recruitment started with groups that had previously published on this topic who met our inclusion criteria. Additional groups, identified through referral by existing consortium members and self-referral based on the publication of our protocol, that had not published on this topic, but which satisfied the inclusion criteria, were also invited to participate. Supplemental Table S1 shows the datasets contributing to this meta-analysis and how they relate to the Risch meta-analysis<sup>23</sup> based on primary data and the three literature-based meta-analyses of Munafò, Karg, and Sharpley.<sup>24-26</sup> The studies contributing to each analysis varied, with no study contributing results for every analysis. Here we cite the foundational papers for the published studies that contributed results to the project.<sup>30-58</sup>

#### **II. Development of the protocol**



The consortium developed an analysis protocol that focused on data harmonization and analysis prioritization. The decision was made to analyze childhood maltreatment as a source of stress separately from other sources of life stress because childhood maltreatment was assumed to precede the initial onset of depression and to have a significant life-long impact.<sup>59-61</sup> Life stressors other than childhood maltreatment include such things as physical or sexual assault, experience of life-threatening illness, loss of employment, loss of a spouse, or military conscription. When possible, analyses of other life stressors included information on the timing of both the stressful events and the depression assessment. For both childhood maltreatment and broadly defined stress (defined as experiencing either childhood maltreatment or other life stress), we examined histories of both lifetime depression and current depression (at the time of assessment). In addition to stress exposure and genotype, sex and age were used as covariates in our analysis models. Subjects assessed between the ages of 21 and 30 were of particular interest because of the possibility that the effect might be strongest at these ages, which is a similar age range to the individuals in the original report.<sup>16</sup>

All analyses were stratified by genetic ancestry. An outline of the primary analyses can be found in Supplemental Table S2 and more detailed descriptions of the planned analyses are provided in our published protocol.<sup>1</sup>

### **III. Analysis script**

The coordinating center at Washington University in St. Louis developed data coding instructions (Supplemental Table S3) based on the protocol and wrote an analysis script in R.<sup>62</sup> Each participating group reformatted their data for the analysis and executed the analysis script locally on their own data. Results from these analyses, including coefficients and standard errors for the primary and secondary analyses as well as demographic information on the data set, were sent to the coordinating center for meta-analysis.

### **IV. Quality control assessments**

*Data coding:* To ensure high quality data, the analysis team at Washington University examined the submitted results for unusual values (e.g., unexpected allele frequencies, sex ratios, stress exposure rates, rates of depression diagnoses, missing values). When unusual values were found, the team worked with the data providers to ensure that the final results accurately reflected their data.

*Poorly fitted models:* For results from a study to be included in a particular meta-analysis, we required a minimum of 50 individuals to be phenotyped for all variables in the model and that the resulting  $|\beta| < 10$  (corresponding to odds ratios (OR) between 1/20 000 and 20 000). Of the results that satisfied both the minimum sample size and restriction on  $\beta$ , all of the OR for the interaction terms were within the more reasonable range of 1/20 to 20.

## **V. Meta-analysis**

Meta-analyses of both the primary and secondary models were performed using the R packages *rmeta*<sup>63</sup> and *metafor*,<sup>64</sup> and SAS.<sup>65</sup> Because of the great variability of the data sources, all meta-analysis results are based on random effects models even though there was little statistical evidence of heterogeneity (see Supplemental Table S4).

## **VI. Models Analyzed**

In keeping with the original report,<sup>16</sup> we tested the following main hypothesis:

The risk of depression displays an interaction between 5-HTTLPR genotype (LL, LS, SS) and exposure to stress: namely, the 5-HTTLPR genotype shows no association to depression in individuals not exposed to stress, but shows a dose response effect (increased risk for more copies of the S allele) in individuals exposed to stress. Our primary genetic coding was additive in the number of copies of the S allele. Our template for analysis is in the form

$$Depression = age + sex + stress + gene + gene \times stress$$

That is, for a dichotomous depression diagnosis,

$$\text{logit}(D) = \beta_0 + \beta_1 \text{age} + \beta_2 \text{sex} + \beta_3 \text{stress} + \beta_4 \text{gene} + \beta_5 (\text{gene} \times \text{stress}).$$

Support for the hypothesis that S alleles are associated with an increased risk for depression in stress-exposed individuals, but not in individuals who are unexposed to stress, would be reflected by an OR > 1 for the gene x stress interaction term. We examined this main hypothesis in multiple settings in an attempt to determine a range of conditions under which the effect might be found. We examined two types of stress (childhood maltreatment, other life stress), two categories of depression (depression during lifetime, current depression), and two age ranges (all ages, young adults between the ages of 21 and 30).

Additional secondary hypotheses (e.g., whether there is a main effect of 5-HTTLPR variation on depression, whether the effect is observed when using a dominant model (LL vs SL or SS), whether the effect would be observed more strongly in a single sex) were also examined to improve our understanding of this complex topic.

Our broadest analyses incorporated information from studies that could not evaluate the full model (e.g., a study with only female subjects, a study with only stress-exposed subjects). These analyses performed logistic regression on pooled genotype counts with contributing study coded as a class variable in the model.

We used the results for the sex and stress terms as positive controls because females and stress-exposed individuals are known to be at increased risk for depression.

## RESULTS

Our participating groups contributed a total of 43 165 total subjects genotyped for 5-HTTLPR and assessed for depression and childhood maltreatment and/or other stressful life events. Of these, 40 693 (94.3%) were of European ancestry, and after harmonization 38 802 subjects contributed to at least one analysis. The non-European samples were distributed across five strata (African, African-European Admixed, Asian, Pacific Islander, and Hispanic) and were not meta-analyzed due to small

sample size. Supplemental Table S5 provides key demographic information about the data included in the meta-analyses (e.g., N, S allele frequency, frequencies of the key phenotypes). For each of the datasets in Table S5, Table S6 lists whether the study design was cross-sectional or longitudinal, the criteria used to diagnose depression, and the assessments used to determine childhood maltreatment and other stressful life events. Table S7 provides information about additional datasets for which the script was run, but whose results could not be included in any of the primary or secondary analyses. Further details about each participating study can be found in Supplemental Table S8.

Table 1 lists results from analyses across all age groups based on exposure to our two stressors of interest and diagnoses of our two depression outcomes. As expected, our two positive control factors, sex (OR < 1, indicating that males are at lower risk) and exposure to stress (OR > 1 indicating that exposure to stress increases risk), each have strong, consistent, and highly statistically significant associations to diagnoses of depression whether the diagnosis was for lifetime depression or current depression at the time of assessment. We do not see a main effect association between number of copies of the S allele and depression in these analyses, a finding that matches what we would expect from prior reports, including the study originally reporting the interaction.<sup>16</sup>

Importantly, our meta-analyses do not support the hypothesis that in subjects exposed to stress, carrying S alleles for 5-HTTLPR confers a differential and increased risk for either lifetime or current depression compared to the impact of carrying S alleles in subjects who were not exposed to stress. In fact, when the outcome is current depression, the point estimates for the interaction terms are all in the direction opposite of the hypothesis.

The broad stress analyses examined stress resulting from either childhood maltreatment or other life stress. The other life stress exposure was examined in two ways: including only subjects for whom the other life stress was documented to have occurred within the five years prior to depression (five years prior to assessment if no depression) or including all subjects. The 5-year threshold was chosen to match the original study design of Caspi et al. (2003).<sup>16</sup> Most of the studies contributing to this set of analyses assessed stress over a shorter period, which is more in line with current beliefs about the

depressogenic effects of acute stressors experienced in adulthood.

Forest plots illustrating how the individual studies contribute to the first meta-analysis in Table 1 (outcome: lifetime depression diagnosis; stress: exposure to childhood maltreatment) are shown in Figure 1. The protective effect of being male (Figure 1A) and the risk from stress (Figure 1B) are consistent across the individual studies, and correspond to overall p-values of  $1.4\text{E-}15$  and  $1.7\text{E-}8$ , respectively. The lack of a main effect for the genetic variant in this model is also consistent across the studies (Figure 1C). For the interaction terms (childhood maltreatment exposure by number of S alleles) (Figure 1D), the point estimates are scattered on both sides of 1, and correspond to an overall p-value of 0.49.

Forest plots for these four key factors (sex, stress, gene, and gene x stress interaction) for the remaining analyses summarized in Table 1 are given in Supplemental Figures S1 through S5. Forest plots for the interaction terms for the remaining primary and secondary analyses are given in Supplemental Figures S6 to S14.

Our other primary analyses and additional secondary analyses examined questions of the strength, robustness, and conditions required to observe the hypothesized interaction. The results presented in Table 1 reflect the general consensus of the findings. None of the other primary analyses (results in Supplemental Tables S9 through S12) or secondary analyses (results in Supplemental Tables S13 through S16) resulted in a statistically significant interaction.

We note that a closely related pair of young adult primary analyses resulted in *nominally* significant interactions (p-value < 0.05 before correction for multiple tests) in the hypothesized direction (Supplemental Table S10b). Several factors caution against placing too much confidence in these particular results: (i) failure of positive control – the point estimate for exposure to stress is protective for depression in these two analyses, counter to our more robust analyses and to what would be expected; (ii) they are not supported by closely related analyses – neither the matching analysis based on childhood maltreatment only, nor the other young adult analyses are even nominally significant (Tables

S10b, S9, and S10a), and the matching analysis with subjects of *all ages* has the point estimates of effect in the opposite direction (Table S11b); (iii) statistical instability – these two analyses only include a small number of studies (3 and 4) with a relatively small total sample size (N=583 and N=1142), and are primarily driven by results from a single study; and (iv) neither p-value survives correction for the number of primary analyses performed.

Our protocol included secondary analyses to help determine whether analytic refinements might strengthen the result and explain why the hypothesized interaction had not heretofore been found consistently. To reduce heterogeneity in depression diagnosis, we examined the effect of restricting meta-analyses to depression diagnoses based on DSM or ICD criteria (Supplemental Table S13). To determine if the interaction might be predominantly expressed in only one sex, we performed meta-analyses stratified by sex (Supplemental Table S14). We examined alternative coding of the genetic effect (dominant, recessive, haplotype) (Supplemental Table S15). Because the question of causation depends on temporal order of events, we examined whether the interaction would be stronger if the analyses were restricted to data from longitudinal studies that had recorded temporal order (Supplemental Table S16). In each case, there is a trade-off between a possible gain in power due to a refined phenotype versus a loss in power due to smaller sample size. For these secondary analyses, we observed one nominally significant interaction in the opposite direction from the hypothesis (Supplemental Table S13, depression diagnosis restricted to DSM or ICD, broad stress, current depression, OR = 0.74,  $p = 0.01$ ), and one nominally significant interaction in the hypothesized direction (Supplemental Table S15b), S allele coded as recessive, broad stress, lifetime depression, OR 1.25,  $p = 0.02$ ). In all other analyses, the interaction term was not even nominally significant.

We evaluated the heterogeneity for the interaction terms in all the previous analyses. Supplemental Table S4 lists the  $I^2$  and Q heterogeneity statistics along with the p-value for the Q statistic for all the primary and secondary meta-analyses in the subsequent tables, demonstrating that there is generally little evidence for heterogeneity in these analyses. In particular, secondary analyses refining the diagnostic criteria and the study design did not substantially decrease the heterogeneity.

Cumulatively, these primary and secondary results exclude a strong, broadly generalizable interaction effect reported in Caspi et al. 2003.<sup>16</sup>

## DISCUSSION

A hallmark of science is the ability of results to be replicated, a criterion that has been increasingly recognized in biological and psychological research.<sup>66</sup> The original 2003 report of an interaction between 5-HTTLPR genotype and stress exposure on depression<sup>16</sup> has remained controversial due to inconsistent results from replication efforts. Although some researchers have claimed a replication of the hypothesized interaction based on different stressors, different measures of depression, or different genetic models,<sup>25, 26</sup> other attempts to replicate the finding have been negative.<sup>23, 24, 67</sup> The goal of our study was to rigorously explore the extent to which the original report could be replicated and generalized using a structured collaborative meta-analysis.

This is the largest study to date to use consistent statistical analyses across all samples to examine the hypothesized interaction between 5-HTTLPR genotype and stress exposure affecting major depression. As detailed in our protocol,<sup>1</sup> our design was based on consistent, de novo analyses chosen by a consensus of participating researchers in the field, with inclusion open to all researchers with published or unpublished data that met objective minimum participation criteria. The purpose was to address multiple issues of concern about previous meta-analyses of the topic: (i) heterogeneity of phenotypes, (ii) publication bias from small studies, (iii) heterogeneity of statistical models used to produce the input for the meta-analysis, (iv) meta-analysis models that did not take direction of effect into account.

Neither our primary nor our secondary analyses found compelling evidence that the 5-HTTLPR S allele increases risk of major depression in individuals exposed to stress. These results are in marked contrast to the robust main effect signals seen for the sex and stress exposure, where p-values less than  $10^{-60}$  were seen in our most inclusive primary analyses (Supplemental Table S12). In our effort to

determine conditions for which the interaction might be reliably detected, we investigated both childhood maltreatment and other life experiences as stressors. Because major depression is a recurring and remitting disease subject to recall bias, both current depression and lifetime depression were examined. Data from subjects of any age and data limited to young adults were both studied. We examined life stress known to precede depression (thereby limiting the sample to studies that documented the relative timing of stress and depression) and we investigated whether the hypothesized interaction could be more effectively detected using all available data with stress and depression assessed. In secondary analyses, we also examined multiple models for the coding of the genotype (additive, dominant, recessive, haplotypes) as well as broad and narrow requirements for documentation of temporal order of the stress experience and the onset of depression. Despite these efforts, we were unable to uncover specific subgroups where the GxE interaction was clearly expressed.

The Caspi group that originally proposed the hypothesis<sup>16</sup> raised concerns regarding this meta-analysis project; in particular, the decisions to exclude small studies, and to include lifetime depression as an outcome for analysis were criticized.<sup>68</sup> As noted in our methods, although we required studies to have at least 300 participants overall, inclusion in any particular meta-analysis required only 50 of these subjects to be genotyped and have the appropriate phenotypes (ancestry, depression outcome, covariates). Although Moffitt and Caspi argue that small studies may be meticulously designed and have high quality data,<sup>68</sup> there is a case to be made that large studies are generally likely to have better design quality than small studies.<sup>69</sup> In addition, small studies are subject to multiple statistical issues, including publication bias (exacerbated for small studies) and the winner's curse (which makes it likely, even if a true effect is detected, that the magnitude will be exaggerated).<sup>70</sup> In fact, a 2013 analysis of neuroscience publications concluded that small sample size studies were undermining the reliability of neuroscience.<sup>69</sup>

The concern Moffitt and Caspi raised regarding the inclusion of lifetime depression analyses was the difficulty of knowing the relative timing of stress and depression for a lifetime phenotype. Rather than



omit these analyses of lifetime depression, as suggested by Moffitt and Caspi, we included analyses where timing information was specifically queried as well as analyses where it was not specifically queried. We recognize that these data, like all data, have limitations, but nonetheless we find the results informative. We note that of all the models examined in our de novo analyses, the only results with nominally significant interaction terms in the hypothesized direction were based on lifetime depression outcomes. Finally, based on parameter estimates provided in the supplement to their seminal paper,<sup>16</sup> we can estimate the impact those data would have on both our young adult and all-age analyses involving depression diagnoses with a quantitative life stress variable. We found that none of these analyses were nominally significant even after adding the Caspi et al. (2003) results to the meta-analyses.

The decision by some invited groups not to participate is a limitation of this project. It is becoming increasingly clear that large samples are an important tool for determining the role of genetic variation in complex phenotypes, such as depression. Combining existing data is an efficient tool for this purpose. We expect that in the future data sharing will become the rule rather than the exception. We are encouraged by the fact that data sharing is becoming a requirement of funding agencies and a requirement for publication by some journals. Although we would have preferred complete participation, several factors mitigate the impact that this likely had on our results. First, the phenotypes for several of the non-participating groups turned out to be insufficient for inclusion in any of our primary or secondary analyses. Second, several of the non-participating groups had exclusively Asian samples, which would not have impacted the European ancestry results. Finally, we found that some data reported in the large prior meta-analyses as supportive of the interaction were not supportive when all were analyzed using the same statistical model for all studies.

Although our consortium tested many high-priority combinations of factors (see Supplemental Table S2), there remain other specific situations that we were unable to evaluate, such as limiting analyses to financial stress,<sup>71</sup> to persistent or recurrent depression,<sup>29, 72</sup> or to childhood emotional abuse/neglect only.<sup>73</sup> Using data from a diverse set of studies, most designed to address other questions, is also a

limitation. However, we note that many of the participating studies, despite their diversity, have already been cited in the literature either in support of, or against, the hypothesized interaction.

Our novel contribution is to apply a consistent methodology across the participating studies to query a broad range of questions about the hypothesized interaction. Although these studies remain varied in their original design, our unified approach to phenotype harmonization and statistical analysis has provided a sound and comprehensive exploration of this challenging question. We have addressed and excluded the major objections (exclusion of small studies, inclusion of analyses of lifetime depression) to our protocol raised by Caspi and Moffitt.

Our findings do not support the interaction hypothesis. We found no subgroups or variable definitions for which an interaction between stress and 5-HTTLPR genotype was statistically significant. In contrast, our findings for the main effects of sex (strong risk factor), life stressors (strong risk factor) and 5-HTTLPR genotype (no impact on risk) are strikingly consistent across the models examined in this study. Moreover, these robust main effect results are consistent with the main effect results from the Caspi study that originally reported the interaction,<sup>16</sup> with the re-examination of the topic using primary data by Risch et al.,<sup>23</sup> and with prior meta-analyses.<sup>23-26</sup> Based on our findings, we conclude that if an interaction exists in which the S allele of 5-HTTLPR increases risk of depression only in stressed individuals, then it is not a broadly generalizable effect, but must be of modest effect size and only observable in limited situations. Our lack of replication coincides with findings of the Christchurch Health and Developmental Study,<sup>67</sup> a prospective longitudinal birth-cohort, with measures, outcomes, and sample (both size and origin on the south island of New Zealand) nearly identical to the original report. This lack of evidence for a strong, robust effect should be taken into account before planning future research on this topic.

Supplementary information is available at *Molecular Psychiatry's* website.

## ACKNOWLEDGMENTS

**Acknowledgment of funding:** Funding supporting the participation of the contributing studies was as follows:

ALSPAC: Grant 102215/2/13/2 from The Wellcome Trust and grant MC\_UU\_12013/6 from the UK Medical Research Council. The University of Bristol also provides core support for ALSPAC. MRM is a member of the UK Centre for Tobacco and Alcohol Studies, a UK Clinical Research Council Public Health Research: Centre of Excellence. Funding from British Heart Foundation, Cancer Research UK, Economic and Social Research Council, Medical Research Council, and the National Institute for Health Research, under the auspices of the UK Clinical Research Collaboration, is gratefully acknowledged.

ASPIS: EKBAN 97 from the General Secretariat of Research and Technology, Greek Ministry of Development.

ATP: Grants DP130101459, DP160103160 and APP1082406 from the Australian Research Council and The National Health and Medical Research Council of Australia.

CHDS: Grant HRC 11/792 from the Health Research Council of New Zealand.

CoFaMS: Grant APP1060524 to BTB from the National Health and Medical Research Council of Australia. The authors wish to acknowledge the University of Adelaide for the provision of seed funding in support of this project.

COGA: Grant U10AA008401 from the National Institutes of Health, NIAAA and NIDA.

COGEN: National Institutes of Health grants P01CA089392 from NCI and R01DA036583 from NIDA.

DeCC: Grant G0701420 from the UK Medical Research Council, and a UK MRC Population Health Scientist fellowship (G1002366) and an MQ Fellows Award (MQ14F40) to Helen L. Fisher.

EPIC-Norfolk: Grants G9502233, G0300128, C865/A2883 from the UK Medical Research Council and Cancer Research UK.

ESPRIT Montpellier: An unconditional grant from Novartis and from the National Research Agency (ANR Project 07 LVIE004).

G1219: A project grant from the WT Grant Foundation and G120/635, a Career Development Award from the UK Medical Research Council to Thalia Eley. The GENESiS project was supported by Grant G9901258 from the UK Medical Research Council. This study presents independent research part-funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

GAN12-France: Research Protocol C0829 from INSERM; Research Protocol GAN12 from Assistance Publique des Hôpitaux de Paris; ANR-11-IDEX- 0004 from Investissements d'Avenir program managed by the ANR, and RTRS Sante Mentale from Fondation FondaMental.

GENESIS: Grant PHRC UF 7653 & ANR NEURO 2007 'GENESIS' from CHU Montpellier & Agence Nationale de la Recherche.

Heart and Soul: Epidemiology Merit Review Program from the Department of Veterans Affairs; National Institutes of Health grant R01HL-079235 from NHLBI; Generalist Physician Faculty Scholars Program from the Robert Wood Johnson foundation; Paul Beeson Faculty Scholars Program from the American Federation for Aging Research; and a Young Investigator Award from the Bran and Behavior Research Foundation.

MARS: Grant LA 733/2-1 from German Research Foundation (DFG) and the Federal Ministry for Education and Research as part of the "National Genome Research Network".

MLS: National Institutes of Health grants R01 AA07065 and R37 AA07065 from NIAAA.

MoodInFlame: Grant EU-FP7-HEALTH-F2-2008-222963 from the European Union.

Muenster Neuroimaging Study: Grant FOR2107, DA1151/5-1 from the German Research Foundation (DFG).

NEWMOOD: Grants LSHM-CT-2004-503474 from Sixth Framework Program of the European Union; KTIA\_NAP\_13-1-2013-0001, KTIA\_13\_NAP-A-II/14 from National Development Agency Hungarian Brain Research Program; KTIA\_NAP\_13-2-2015-0001 from MTA-SE-NAP B Genetic Brain Imaging Migraine Research Group, Hungarian Academy of Sciences, Semmelweis University; support from Hungarian Academy of Sciences, MTA-SE Neuropsychopharmacology and Neurochemistry Research Group; and support from the National Institute for Health Research Manchester Biomedical Research Centre

NESDA/NTR: The Netherlands Organization for Scientific Research (NWO) and MagW/ZonMW grants Middelgroot-911-09-032, Spinozapremie 56-464-14192, Geestkracht program of the Netherlands Organization for Health Research and Development (ZonMW 10-000-1002), Center for Medical Systems Biology (CSMB, NWO Genomics), Genetic influences on stability and change in psychopathology from childhood to young adulthood (ZonMW 912-10-020), NBIC/BioAssist/RK(2008.024), Biobanking and Biomolecular Resources Research Infrastructure (BBMRI -NL, 184.021.007), VU University's Institute for Health and Care Research (EMGO+ ) and Neuroscience Campus Amsterdam (NCA); the European Science Council (ERC Advanced, 230374). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health, Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA) and the National Institutes of Health (NIH R01 HD042157-01A1, MH081802, Grand Opportunity grants 1RC2 MH089951 and 1RC2 MH089995).

PATH: Program Grant Number 179805 from the National Health and Medical Research Council of Australia.

POUCH: Grants 20FY01-38 and 20-FY04-37 of the Perinatal Epidemiologic Research Initiative Program Grant from the March of Dimes Foundation; National Institutes of Health grant R01 HD34543 from NICHD and NINR; grant 02816-7 from the Thrasher Research Foundation; and grant U01 DP000143-01 from the Centers for Disease Control and Prevention.

QIMRtwin: Grants 941177, 971232, 339450, 443011 from the National Health and Medical Research Council of Australia; AA07535, AA07728, AA10249 from US Public Health Service; National Institutes of Health grant K99DA023549-01A2 from NIDA. Additional support was provided by Beyond Blue.

SALVe 2001 and SALVe 2006: Grants FO2012-0326, FO2013-0023, FO2014-0243 from The Brain Foundation (Hjärnfonden); SLS-559921 from Söderström-Königskas Foundation; 2015-00897 from Swedish Council for Working Life and Social Research; and M15-0239 from Åke Wiberg's Foundation. Additional funding was provided by Systembolagets Råd för Alkoholforskning, SRA and Svenska Spel Research Council.

SEBAS: National Institutes of Health grants R01 AG16790, R01 AG16661, and R56 AG01661 from NIA and grant P2CHD047879 from NICHD; and additional financial support from the Graduate School of Arts and Sciences at Georgetown University.

SHIP/TREND: This work was supported by the German Federal Ministry of Education and Research within the framework of the e:Med research and funding concept (Integument) Grant No. 01ZX1314E. Study of Health in Pomerania is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research Grant Nos. 01ZZ9603, 01ZZ0103, and 01ZZ0403; the Ministry of Cultural Affairs; and the Social Ministry of the Federal State of Mecklenburg-West Pomerania. Genome-wide data were supported by the Federal Ministry of Education and Research Grant No. 03ZIK012 and a joint grant from Siemens Healthcare, Erlangen, Germany, and the Federal State of Mecklenburg-West Pomerania. The Greifswald Approach to Individualized Medicine (GANI\_MED) was funded by the Federal Ministry of Education and Research Grant No. 03IS2061A and the German Research Foundation Grant No. GR 1912/5-1.

TRAILS: Grants GB-MW 940-38-011, ZonMW Brainpower 100-001-004, Investment grant 175.010.2003.005, GB-MaGW 480-07-001, and Longitudinal Survey and Panel Funding 481-08-013

from the Netherlands Organization for Scientific Research (NWO). Additional funding was provided by the Dutch Ministry of Justice, the European Science Foundation, BBMRI-NL, and the participating centers (UMCG, RUG, Erasmus MC, UU, Radboud MC, Parnassia Bavo group):

VAHCS: Grants APP1063091,1008271, and 1019887 from Australia's National Health and Medical Research Council of Australia (NHMRC).

The coordinating team: National Institutes of Health grants R21 DA033827 and R01 DA026911 from NIDA.

## **Other acknowledgments:**

The authors wish to thank the following for making the participation of each group possible:

ALSPAC: All the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

ATP: All collaborators who have contributed to the Australian Temperament Project, especially Professors Ann Sanson, Margot Prior, Frank Oberklaid, John Toumbourou and Ms Diana Smart. We would also like to sincerely thank the participating families for their time and invaluable contribution to the study. The ATP study is located at The Royal Children's Hospital Melbourne (Australia) and is a collaboration between Deakin University, The University of Melbourne, the Australian Institute of Family Studies, The University of New South Wales, The University of Otago (New Zealand), and the Royal Children's Hospital; further information available at [www.aifs.gov.au/atp](http://www.aifs.gov.au/atp). The views expressed in this paper are those of the authors and may not reflect those of their organisational affiliations, nor of other collaborating individuals or organisations.

CHDS: Allison L. Miller, laboratory team leader, Carney Centre for Pharmacogenomics.

CoFaMS: The authors would like to thank study participants for their time and willingness to take part in the study.

COGA: The Collaborative Study on the Genetics of Alcoholism (COGA), Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes eleven different centers: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, J. Rice, K. Bucholz, A. Agrawal); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield, A. Brooks); University of Texas Rio Grand Valley (L. Almasy), Virginia Commonwealth University (D. Dick), Icahn School of Medicine at Mount Sinai (A. Goate), and Howard University (R. Taylor). Other COGA collaborators include: L. Bauer (University of Connecticut); J. McClintick, L. Wetherill, X. Xuei, Y. Liu, D. Lai, S. O'Connor, M. Plawecki, S. Lourens (Indiana University); G. Chan (University of Iowa; University of Connecticut); J. Meyers, D. Chorlian, C. Kamarajan, A. Pandey, J. Zhang (SUNY Downstate); J.-C. Wang, M. Kapoor, S. Bertelsen (Icahn School of Medicine at Mount Sinai); A. Anokhin, V. McCutcheon, S. Saccone (Washington University); J. Salvatore, F. Aliev, B. Cho (Virginia Commonwealth University); and Mark Kos (University of Texas Rio Grand Valley). A. Parsian and M. Reilly are the NIAAA Staff Collaborators. We thank John Budde, Maribel Martinez, and Oliver Reyes from Alison Goate's lab for their technical assistance in genotyping.

COGEND: COGEND is a collaborative research group and a part of the NIDA Genetics Consortium. Lead investigators who directed data collection include Laura Bierut, Naomi Breslau, Dorothy Hatsukami, and Eric Johnson. The authors thank Heidi Kromrei and Tracey Richmond for their assistance in data collection. We thank John Budde, Maribel Martinez, and Oliver Reyes from Alison Goate's lab for their technical assistance in genotyping.

DeCC: All individuals who participated in the DeCC and BaCCs studies and were essential for their successful completion. Specifically, we acknowledge the leadership of Peter McGuffin and Anne Farmer, and the contribution of the following: Cerisse Gunasinghe, Katharine Mead, Joanna Gray, Ylva Dahlin, Amanda Elkin, Audrey Morgan, Joanna O'Leary, Nathan O'Neill, Nicola Reynolds, Zainab Samaan, Abraham Stern, Linda Southwick, Kopal Tandon, Alison Wheatley, Richard Williamson, Debra Woolway, Julia Woods, Sarah Ball, Ophelia Beer, Julian Childs, Sam Keating, Rachel Marsh, Penny Machin, and Lucy Maddox, who were involved with the data collection and management of the studies.

EPIC-Norfolk: All participants, general practitioners and the EPIC-Norfolk study team for their contribution to the research program.

G1219: The families of the G1219 study for their time, Robert Plomin, Ian Craig, Pak Sham, Karen Sugden, Alice Gregory and Alejandro Crosico, Matthew Nash, Abram Sterne, Richard Williamson and

Maria Napolitano for their contributions to the project.

GAN12-France: Marion Leboyer, Chantal Henry, Stéphane Jamain, Jean-Pierre Kahn, Sébastien Gard. The GAN12-France study includes three centres : Creteil (M. Leboyer, C. Henry), Bordeaux (S. Gard) and Nancy (J.P. Kahn) and also the INSERM U955 Translational Psychiatry research team (S. Jamain).

GENESIS: Catherine Genty and Aurélie Cazals for their assistance in conducting the study.

MARS: The participants and their parents for their engagement in the study. We thank Erika Hohm and Katrin Zohsel for their assistance in conducting the study.

MLS: Robert Zucker for allowing access to the MLS data.

NEWMOOD: Diana Chase, Emma J. Thomas, Darragh Downey, Dorottya Pap, Judit Lazary, Zoltan G. Toth for their assistance in the recruitment and data acquisition and Hazel Platt for her assistance in genotyping.

POUCH: Participants in the POUCH Study and the Project Director, Bertha Bullen, and Nicole Jones.

QIMRtwin: The staff from the Queensland Institute of Medical Research who were involved in the genotyping, the twins (drawn from the Australian NH&MRC Twin Registry) for their cooperation and Professors Ian Hickie, Grant Montgomery, and Naomi Wray for their encouragement.

SALVe 2001: Professor Lars Orelund, Professor Jerzy Leppert, Professor Leif Lindström, Professor John Öhrvik, associate professor Rickard L. Sjöberg and PhD Per-Olof Alm

SEBAS: The staff at the Surveillance and Research Division (Health Promotion Administration at the Ministry of Health and Welfare in Taiwan) who were instrumental in the design and implementation of the SEBAS and supervised all aspects of the fieldwork and data processing.

SHIP/TREND: The participants taking part in the SHIP and TREND study. The contribution to data collection performed by study nurses, study physicians, interviewers, and laboratory workers is gratefully acknowledged. We are also appreciative of the important support of IT and computer scientists, health information managers, and administration staff.

TRAILS: Everyone who participated in this research and made it possible.

U Bologna: Dr. Raffaella Calati for contribution in paper writing, Dr. Elena Marino and Dr. Adele Pirovano for their contribution to genetic analyses, and Prof. Cristina Colombo from the Scientific institute San Raffaele of Milan, Department of Psychiatry, for her precious supervision of the study

U. Molise: Dr. Vladimir Carli (Karolinska Institutet, Stockholm, Sweden), Dr. Leonardo Zaninotto and Prof. Alessandro Serretti (University of Bologna, Italy), Dr. Laura Recchia (University of Molise, Campobasso, Italy), Dr. Valentina Gatta and Dr. Liborio Stoppia (G. d'Annunzio University, Chieti-Pescara, Italy) for their valuable contribution to the study.

The coordinating team: Sherri Fisher, James Childress, Brandie Thurman for administrative support and Katharina Domschke for contributions to the design of the analysis plan.

### **Conflicts of Interest and Disclosures:**

IM Anderson has received consultancy/speaking fees from Servier, Alkermes, Lundbeck/Otsuka, Takeda and Janssen, and grant support from Servier and AstraZeneca.

V Arolt declares that over the last three years he has received compensations for his contributions as member of advisory boards and for presentations for the following companies: Astra-Zeneca, Eli Lilly, Janssen-Organon, Lundbeck, Otsuka, Servier, and Trommsdorff. These co-operations have no relevance to the work that is covered in the manuscript.

T Banaschewski served in an advisory or consultancy role for Hexal Pharma, Lilly, Medice, Novartis, Otsuka, Oxford outcomes, PCM scientific, Shire and Viforpharma. He received conference attendance support and conference support or received speaker's fees from Lilly, Medice, Novartis and Shire. He

is/has been involved in clinical trials conducted by Lilly, Shire & Viforpharma.

F Bellivier has received honoraria or research or educational conference grants from Bristol-Myers Squibb, Otsuka, Eli Lilly & Co., Servier, Takeda, Sanofi Aventis, Lundbeck, AstraZeneca, the European Space Agency and has received peer review research funding from French Ministry of research, Assistance Publique – Hôpitaux de Paris, the National Institute for Research (INSERM) and the NARSAD.

LJ Bierut, AM Goate, and JC Wang are listed as inventors on Issued U.S. Patent 8,080,371, “Markers for Addiction” covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction. NL Saccone is the spouse of SF Saccone, who is also listed as an inventor on the patent.

JFW Deakin variously performed consultancy, speaking engagements, and research for Bristol-Myers Squibb, AstraZeneca, Eli Lilly, Schering Plough, Janssen-Cilag, and Servier (all fees are paid to the University of Manchester to reimburse them for the time taken); he has share options in P1vital.

JI Nurnberger is an investigator for Assurex and Janssen and a consultant for Janssen.

The remaining authors declare no potential conflicts of interest: TM Air, KJ Anstey, R Araya, C Åslund, G Bagdy, BT Baune, F Bellivier, DI Boomsma, L Bowes, M Burmeister, R Burns, EM Byrne, C Coffey, S Cohen-Woods, P Courtet, WF Coventry, RC Culverhouse, U Dannlowski, EJC de Geus, T Eley, S Easteal, B Etain, DM Fergusson, HL Fisher, K Gawronski, D Gleib, N Goldman, X Gonda, HJ Grabe, S Guillaume, A Hatzimanolis, C Holzman, AC Horton, J Horwood, JJ Hottenga, I Jaussent, C Jawahar, C Jennen-Steinmetz, EO Johnson, G Juhasz, M Kennedy, JR Kramer, M Lajnef, M Laucht, KJ Lester, K Little, Y Ma, L Mandelli, NG Martin, CM Middeldorp, M Munafò, M Nauck, E Nederhof, KW Nilsson, AJ Oldehinkel, E Olié, C Olsson, J Ormel, C Otte, GC Patton, BWJH Penninx, P Petschner, WJ Peyrot, K Ritchie, M Sarchiapone, JM Scheid, C Schwahn, A Serretti, G Sinnamon, JH Smit, D Stacey, NC Stefanis, PG Surtees, Y Tian, C Toben, S Van der Auwera, S Villafuerte, H Völzke, N Wainwright, M Weinstein, M Whooley, G Willemsen, HM zu Schwabedissen



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## Tables and Figures:

### Figure 1 legend:

**Figure 1:** Forest plots for lifetime depression diagnosis in subjects of all ages based on exposure to childhood maltreatment as the stressor

$$\text{MODEL: } \textit{depression} = \beta_0 + \beta_1(\textit{age}) + \beta_2(\textit{sex}) + \beta_3(\textit{stress}) + \beta_4(\textit{gene}) + \beta_5(\textit{gene} \times \textit{stress})$$

Depression = lifetime depression diagnosis (never depressed = 0; ever depressed = 1)

Sex (female = 0; male = 1)

Stress = childhood maltreatment (not exposed = 0; exposed = 1)

Gene (additive coding in number of S alleles for 5-HTTLPR (LL = 0; LS=1; SS=2))



**Table 1.** Meta-analysis of the impact of a stress-by-5-HTTLPR genotype interaction on depression based on new, uniform analyses of harmonized dichotomous phenotypes in subjects of all ages

*Childhood Maltreatment*

Depression	Stress	Studies	Subjects	Covariate	OR	95% CI	p-value
Lifetime	Childhood maltreatment	18	21135	Sex	0.57	(0.50 , 0.66)	1.4E-15
				Stress	2.16	(1.65 , 2.82)	1.7E-08
				Gene	1.00	(0.95 , 1.05)	0.95
				Gene x stress	1.05	(0.91 , 1.21)	0.49
Current	Childhood maltreatment	13	13956	Sex	0.63	(0.51 , 0.78)	3.5E-05
				Stress	2.87	(1.87 , 4.41)	1.5E-06
				Gene	1.00	(0.92 , 1.10)	0.97
				Gene x stress	0.93	(0.76 , 1.14)	0.50

*Broad Stress*

Depression	Stress	Studies	Subjects	Covariate	OR	95% CI	p-value
Lifetime	Broad stress (Other life stress < 5 years prior or childhood maltreatment)	19	21938	Sex	0.58	(0.51 , 0.67)	2.8E-15
				Stress	1.82	(1.39 , 2.39)	1.4E-05
				Gene	1.00	(0.95 , 1.06)	0.95
				Gene x stress	1.06	(0.93 , 1.20)	0.40
Current	Broad stress (Other life stress < 5 years prior or childhood maltreatment)	14	13835	Sex	0.63	(0.51 , 0.78)	2.4E-05
				Stress	3.19	(2.08 , 4.91)	1.2E-07
				Gene	1.01	(0.90 , 1.12)	0.91
				Gene x stress	0.92	(0.76 , 1.11)	0.39
Lifetime	Broad stress (Other life stress or childhood maltreatment)	21	28252	Sex	0.60	(0.53 , 0.67)	6.5E-17
				Stress	2.00	(1.56 , 2.56)	3.8E-08
				Gene	1.00	(0.94 , 1.07)	0.92
				Gene x stress	1.05	(0.94 , 1.16)	0.38
Current	Broad stress (Other life stress or childhood maltreatment)	17	17015	Sex	0.61	(0.49 , 0.75)	5.1E-06
				Stress	2.60	(1.62 , 4.19)	7.8E-05
				Gene	1.08	(0.92 , 1.27)	0.35
				Gene x stress	0.85	(0.68 , 1.07)	0.17

In this table the **childhood maltreatment** analyses represent Primary Analysis 2Ai from the hierarchy presented in Supplemental Table S2. The **broad stress** analyses represent Primary Analysis 2Bi from the hierarchy.

Sex (female = 0; male = 1)

Stress (not exposed = 0; exposed = 1)

Gene (additive coding in number of S alleles for 5-HTTLPR (LL = 0; LS=1; SS=2))

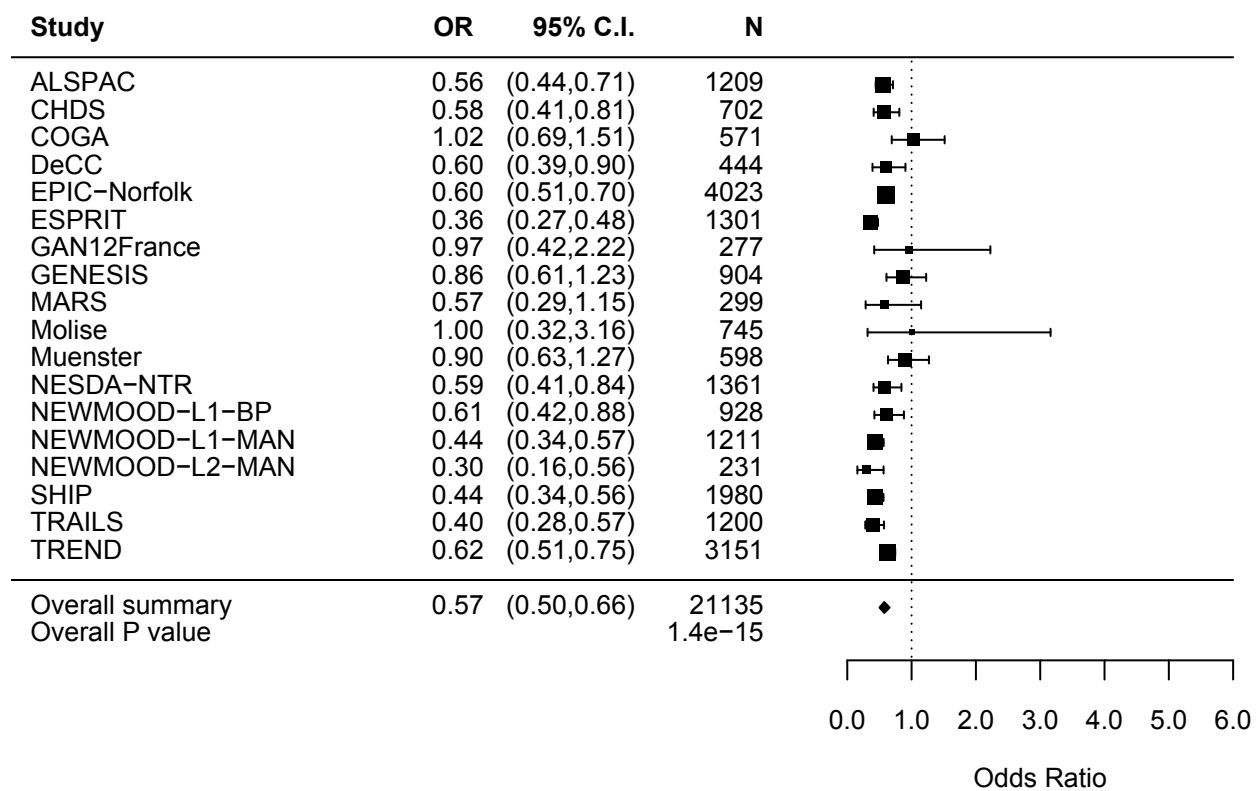
Broad stress does not require both stressors to be assessed.

MODEL:  $depression = \beta_0 + \beta_1(age) + \beta_2(sex) + \beta_3(stress) + \beta_4(gene) + \beta_5(gene \times stress)$

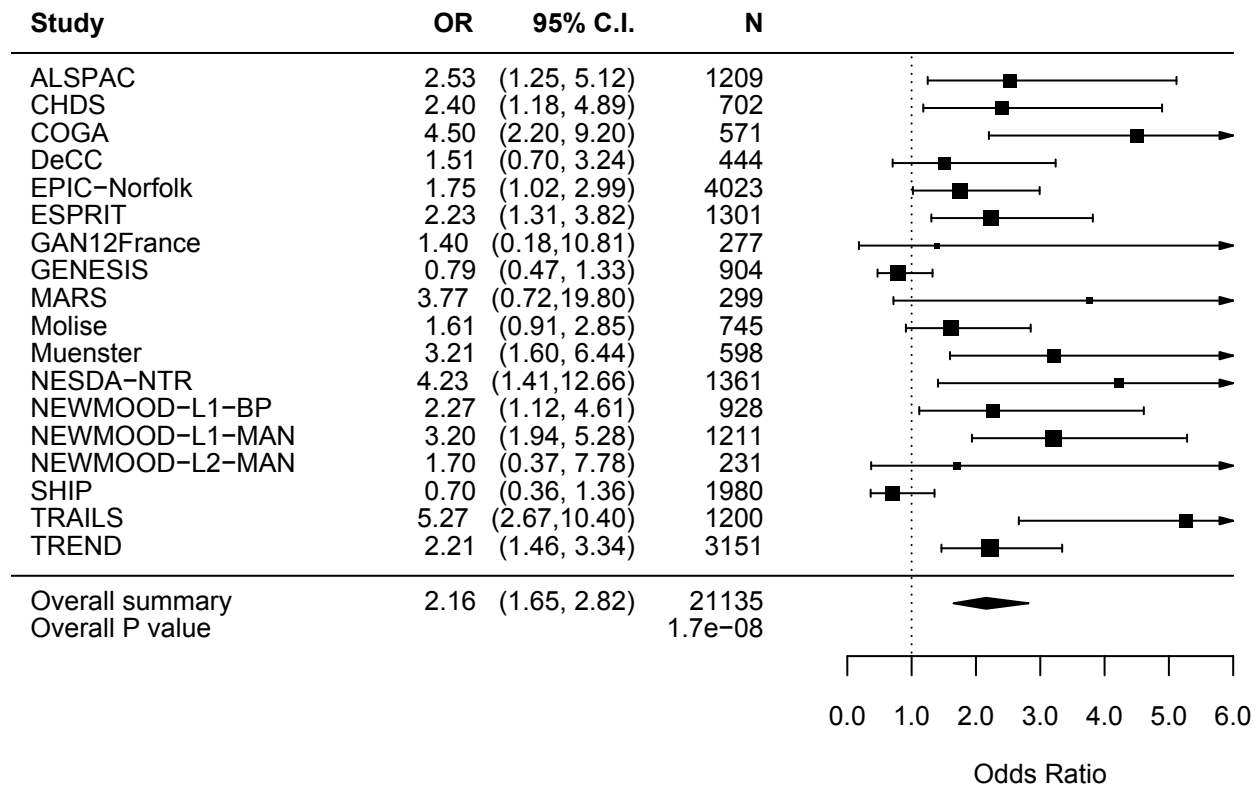
Depression variable: **depression diagnosis**

Stress variable: **dichotomous stress exposure**

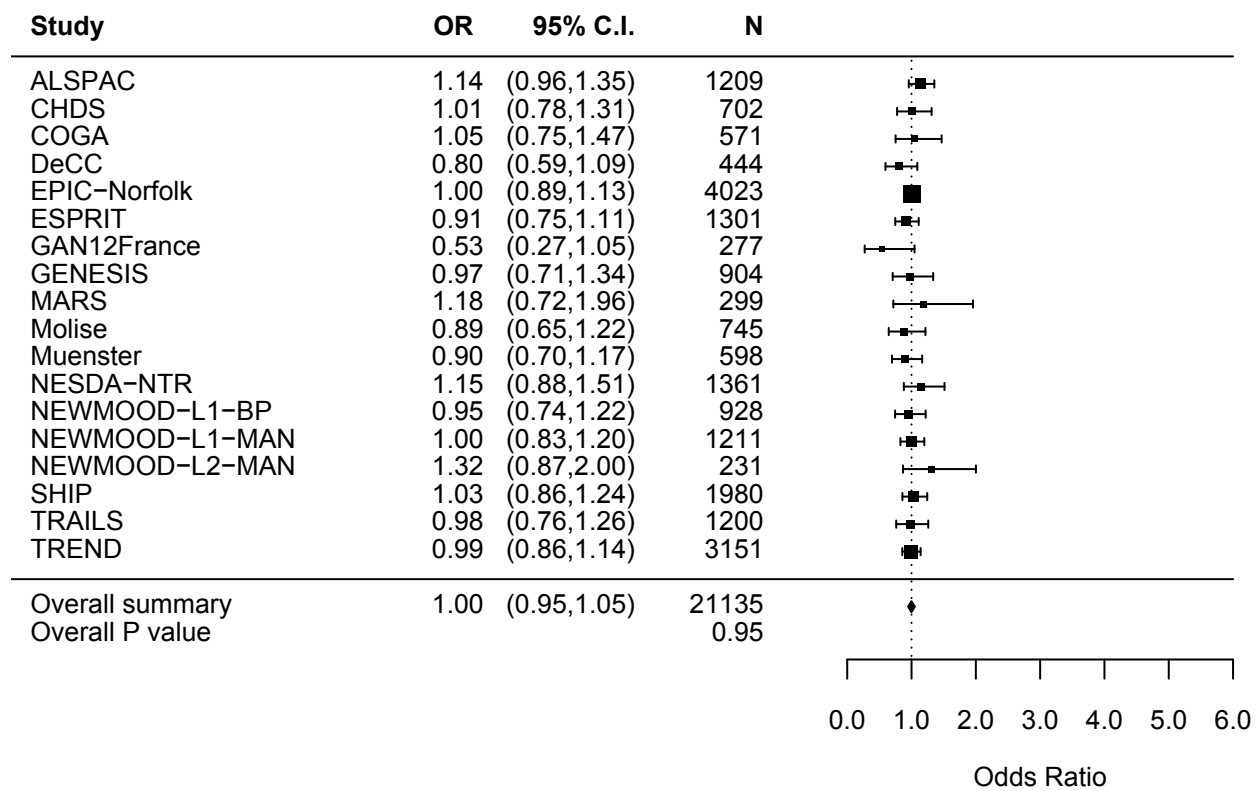
Age was not significant in any of the models



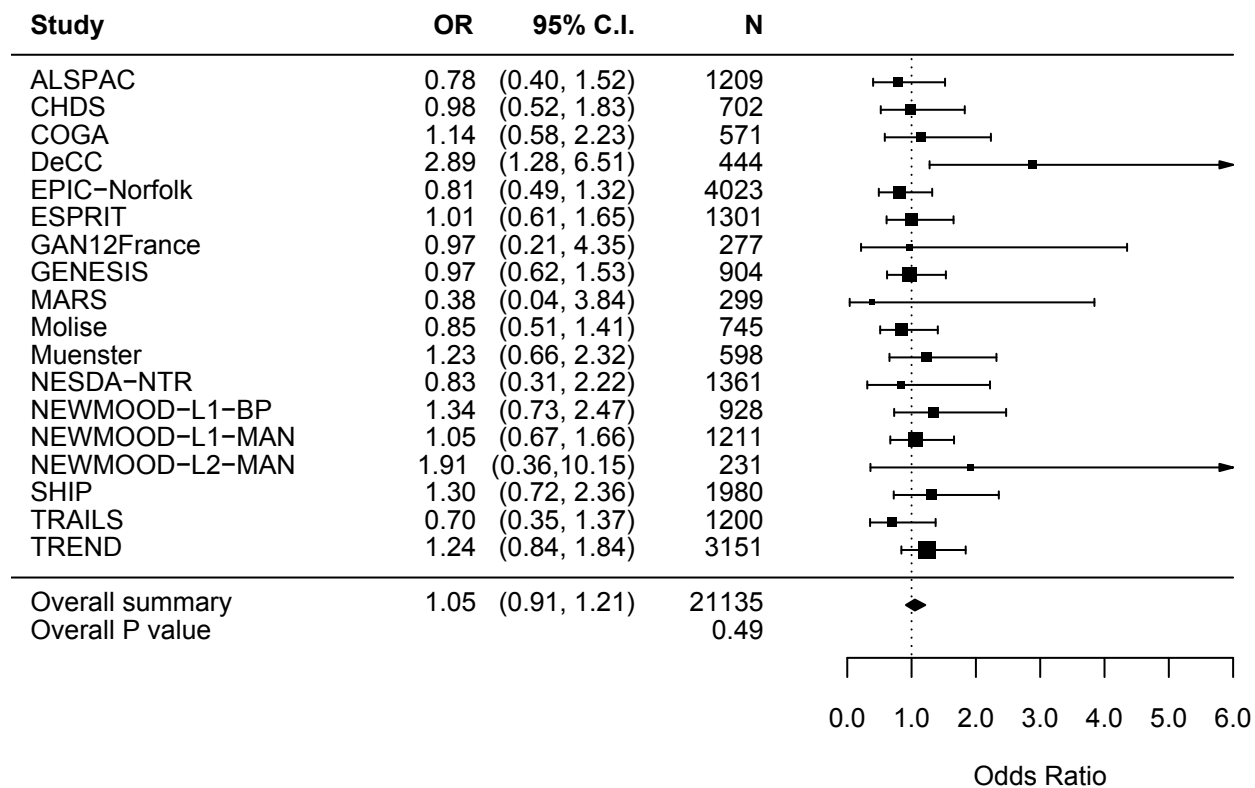
1a. **Sex:** Being male consistently and significantly protects from a lifetime diagnosis of depression.



1b. **Stress:** Exposure to stress consistently and significantly increases lifetime risk for depression.



1c. **Gene:** The S allele (coded additively) is not associated with risk of lifetime depression.



1d. **Gene x Stress:** Interaction term is not significant and does not suggest a consistent direction of effect across studies.  
(Hypothesized direction of effect: OR > 1)