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

A full list of authors and affiliations appears at the end of the article.

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members or self-identified in response to our protocol (published prior to the start of analysis¹) with qualifying unpublished data were also invited to participate.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 metaanalyses. 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 disease² and is a leading cause of total disease burden worldwide.³ Both genetic and environmental factors influence depression; 4 research on the etiology of depression suggests substantial heritability of 40-50%. 4-9 Only recently have genome-wide association studies (GWAS) begun to identify and replicate specific loci associated with depression. 10-12 The findings from these studies suggest that (1) the effects of individual single nucleotide polymorphisms (SNPs) on major

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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. ¹³ Gene-environment interactions (G×E) (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. ^{14, 15}

A high profile report of a G×E 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. ¹⁶ *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. ^{17, 18} 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 G×E 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 G×E interaction continues. Although it is likely that G×E 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. ¹⁹ Furthermore, many candidate gene main-effect association reports appear to be false positives. ^{20, 21} As Duncan and Keller²² 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, ^{23–26} with various reasons proposed for the differences. ^{22, 25, 27–29} Munafò et al.²⁴ performed a literature-based meta-analysis, finding that only 5 of the previously published studies (N = 2999) 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.²³ 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. 25 (56 studies, N= 40 749) and Sharpley et al. 26 (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.²² One key issue contributing to disputes over the

appropriateness of the prior reports and meta-analyses is the heterogeneity of the studies.²² 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. 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-analysis²³ based on primary data and the three literature-based meta-analyses of Munafò, Karg, and Sharpley. 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. 30–58
- **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. ^{59–61} 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.¹⁶

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. All code and documents relevant for running the analyses and meta-analysis are available in the public repository at https://github.com/achorton/SD_5HTTLPR.

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.⁶² 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 metanalysis.

IV. Quality control assessments

<u>Data coding:</u> 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 β , 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 rmeta⁶³ and metafor,⁶⁴ and SAS.⁶⁵ 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, ¹⁶ 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,

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

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 \times 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. 16

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). 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 1.4E–15 and 1.7E–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 × 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. 2003.¹⁶

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. The original 2003 report of an interaction between 5-HTTLPR genotype and stress exposure on depression 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, 25, 26 other attempts to replicate the finding have been negative. 23, 24, 67 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, 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 G×E interaction was clearly expressed.

The Caspi group that originally proposed the hypothesis ¹⁶ 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. ⁶⁸ 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, ⁶⁸ there is a case to be made that large studies are generally likely to have better design quality than small studies. ⁶⁹ 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). ⁷⁰ In fact, a 2013 analysis of neuroscience publications concluded that small sample size studies were undermining the reliability of neuroscience. ⁶⁹

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, ¹⁶ 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 stress over a period shorter than five years, to financial stress, ⁷¹ to persistent or recurrent depression, ^{29, 72} or to childhood emotional abuse/neglect only. ⁷³ 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, ¹⁶ with the re-examination of the topic using primary data by Risch et al., ²³ and with prior meta-analyses. ^{23–26} 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, ⁶⁷ 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 Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Robert C. Culverhouse¹, Nancy L. Saccone², Amy C. Horton³, Yinjiao Ma³, Kaarin J. Anstey⁴, Tobias Banaschewski⁵, Margit Burmeister^{6,7}, Sarah Cohen-Woods⁸, Bruno Etain^{9,10,11}, Helen L. Fisher¹², Noreen Goldman¹³, Sébastien Guillaume^{14,15,16}, John Horwood¹⁷, Gabriella Juhasz^{18,19,20}, Kathryn J. Lester²¹, Laura Mandelli²², Christel M. Middeldorp^{23,24}, Emilie Olié^{14,15,16}, Sandra Villafuerte⁶, Tracv M. Air²⁵, Ricardo Arava²⁶, Lucy Bowes²⁷, Richard Burns⁴, Enda M. Byrne²⁸, Carolyn Coffey²⁹, William L. Coventry³⁰, Katerina Gawronski³¹, Dana Glei³², Alex Hatzimanolis³³, Jouke-Jan Hottenga^{23,34}, Isabelle Jaussent¹⁵, Catharine Jawahar²⁵, Christine Jennen-Steinmetz³⁵, John R. Kramer³⁶, Mohamed Lajnef³⁷, Keriann Little^{38,39}, Henriette Meyer zu Schwabedissen⁴⁰, Matthias Nauck⁴¹. Esther Nederhof⁴², Peter Petschner^{19,43}, Wouter J. Peyrot⁴⁴, Christian Schwahn⁴⁵, Grant Sinnamon²⁵, David Stacey²⁵, Yan Tian⁴⁶, Catherine Toben²⁵, Sandra Van der Auwera⁴⁷, Nick Wainwright⁴⁸, Jen-Chyong Wang⁴⁹, Gonneke Willemsen^{23,34}, Ian M. Anderson^{20,50}, Volker Arolt⁵¹, Cecilia Åslund^{52,53}, Gyorgy Bagdy^{19,43}, Bernhard T. Baune²⁵, Frank Bellivier^{9,10,11}, Dorret I. Boomsma^{23,24,34}, Philippe Courtet^{14,15,16}, Udo Dannlowski^{51,54}, Eco J.C. de Geus^{23,34}, John F. W.

Deakin^{20,50}, Simon Easteal⁵⁵, Thalia Eley⁵⁶, David M. Fergusson¹⁷, Alison M. Goate⁴⁹, Xenia Gonda^{19,43,57}, Hans J. Grabe⁴⁷, Claudia Holzman⁴⁶, Eric O. Johnson⁵⁸, Martin Kennedy⁵⁹, Manfred Laucht⁵, Nicholas G. Martin⁶⁰, Marcus Munafò^{61,62}, Kent W. Nilsson^{52,53}, Albertine J. Oldehinkel⁴², Craig Olsson^{39,63,64}, Johan Ormel⁴², Christian Otte⁶⁵, George C. Patton⁶⁶, Brenda W.J.H. Penninx⁴⁴, Karen Ritchie¹⁵, Marco Sarchiapone⁶⁷, JM Scheid⁶⁸, Alessandro Serretti²², Johannes H. Smit⁴⁴, Nicholas C. Stefanis³³, Paul G. Surtees⁴⁸, Henry Völzke⁶⁹, Maxine Weinstein³², Mary Whooley⁷⁰, John I. Nurnberger Jr⁷¹, Naomi Breslau⁴⁶, and Laura J. Bierut³

Affiliations

¹Department of Medicine and Division of Biostatistics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA ²Department of Genetics and Division of Biostatistics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA ³Department of Psychiatry, Washington University in St. Louis School of Medicine, St. Louis, MO, USA ⁴Centre for Research on Ageing, Health and Wellbeing, The Australian National University, Canberra, Australia ⁵Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany ⁶Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA ⁷Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA 8Discipline of Psychiatry, Flinders University, Adelaide, Australia 9Sorbonne Paris Cité, Université Paris Diderot, UMR-S 1144, Paris, France ¹⁰AP-HP, Groupe Saint-Louis-Lariboisière-F. Widal, Paris, France ¹¹INSERM, U1144, Paris, France ¹²Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK ¹³Office of Population Research, Princeton University, Princeton, NJ, USA ¹⁴Université Montpellier; Montpellier, France ¹⁵INSERM U1061 Neuropsychiatry, Montpellier, France ¹⁶Department of Emergency Psychiatry and Acute Care, CHU Montpellier, Montpellier, France ¹⁷Department of Psychological Medicine, University of Otago Christchurch, Christchurch, New Zealand ¹⁸MTA-SE-NAP B Genetic Brain Imaging Migraine Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary ¹⁹Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary ²⁰Neuroscience and Psychiatry Unit, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK ²¹School of Psychology, University of Sussex, Brighton, UK ²²Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy ²³Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands ²⁴Neuroscience Campus Amsterdam, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands ²⁵Discipline of Psychiatry, University of Adelaide, Adelaide, Australia ²⁶Centre for Global Mental Health, London School of Hygiene and Tropical Medicine, London, UK ²⁷Department of Experimental Psychology, University of Oxford, Oxford, UK ²⁸Queensland Brain Institute, University of Queensland, Brisbane, Australia ²⁹Centre for Adolescent Health, Murdoch Childrens Research Institute, Melbourne, Australia 30 Discipline of Psychology, University of

New England, Adelaide, Australia ³¹Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA 32Center for Population and Health, Georgetown University, Washington, DC, USA 33 University Mental Health Research Institute and Department of Psychiatry, University of Athens School of Medicine, Athens, Greece ³⁴EMGO+ institute for Health and Care Research, VU Medical Center Amsterdam, Amsterdam, the Netherlands 35Department of Biostatistics, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany ³⁶Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA, USA ³⁷INSERM U955, Creteil, France ³⁸Murdoch Childrens Research Institute, Melbourne, Australia ³⁹Department of Paediatrics and School of Psychological Sciences, University of Melbourne, Melbourne, Australia ⁴⁰Biopharmacy, Department Pharmaceutical Sciences, University of Basel, Basel, Switzerland ⁴¹Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany ⁴²University of Groningen, University Medical Center Groningen, Interdisciplinary Center Psychopathology and Emotion Regulation, Groningen, the Netherlands ⁴³MTA-SE Neuropsychopharmacology and Neurochemistry Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary ⁴⁴Department of Psychiatry, VU University Medical Center & GGZ inGeest, Amsterdam, the Netherlands ⁴⁵Department of Prosthetic Dentistry, Gerostomatology and Dental Materials, University Medicine Greifswald, Greifswald, Germany ⁴⁶Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA ⁴⁷Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany ⁴⁸Department of Public Health and Primary Care, School of Clinical Medicine, Cambridge, UK ⁴⁹Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA ⁵⁰Manchester Academic Health Sciences Centre, Manchester, UK ⁵¹Department of Psychiatry and Psychotherapy, University of Münster, Münster, Germany ⁵²Centre for Clinical Research, Uppsala University, Uppsala, Sweden 53Västmanland County Hospital Västerås, Västerås, Sweden ⁵⁴Department of Psychiatry, University of Marburg, Marburg, Germany ⁵⁵John Curtin School of Medical Research, The Australian National University, Canberra, Australia 56King's College London, Institute of Psychiatry, Psychology & Neuroscience, London, UK ⁵⁷Department of Psychiatry and Psychotherapy, Kutvolgyi Clinical Center, Semmelweis University, Budapest, Hungary ⁵⁸Fellow Program and Behavioral Health and Criminal Justice Division, RTI International, Research Triangle Park, NC, USA ⁵⁹Department of Pathology. University of Otago Christchurch, Christchurch, New Zealand ⁶⁰Genetic Epidemiology, QIMR Berghofer, Brisbane, Australia 61MRC Integrative Epidemiology Unit at the University of Bristol, Bristol, UK 62UK Centre for Tobacco and Alcohol Studies, School of Experimental Psychology, University of Bristol, Bristol, UK ⁶³Centre for Social and Early Emotional Development, School of Psychology, Faculty of Health, Deakin University, Geelong, Australia ⁶⁴Centre for Adolescent Health, Murdoch Childrens Research Institute, Melbourne, Australia 65Charité Universitätsmedizin Berlin, Klinik für Psychiatrie und Psychotherapie Campus

Benjamin Franklin, Berlin, Germany ⁶⁶Department of Paediatrics, Murdoch Childrens Research Institute, University of Melbourne, Melbourne, Australia ⁶⁷Department of Health Sciences, University of Molise, Campobasso, Italy ⁶⁸Department of Psychiatry, Michigan State University, East Lansing, MI, USA ⁶⁹Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany ⁷⁰Veterans Affairs Health Care System and University of California, San Francisco, CA, USA ⁷¹Institute of Psychiatric Research, Departments of Psychiatry and Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

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Study	OR	95% C.I.	N	
ALSPAC	0.56	(0.44, 0.71)	1209	•
CHDS	0.58	(0.41, 0.81)	702	H ≣ H :
COGA	1.02	(0.69,1.51)	571	_
DeCC EPIC-Norfolk	0.60 0.60	(0.39,0.90) (0.51,0.70)	444 4023	
ESPRIT	0.36	(0.27,0.48)	1301	
GAN12France	0.97	(0.42, 2.22)	277	
GENESIS	0.86	(0.61, 1.23)	904	+■-
MARS	0.57	(0.29,1.15)	299	- ■ : : :
Molise	1.00	(0.32, 3.16)	745	⊢
Muenster	0.90	(0.63, 1.27)	598	⊢≣ →
NESDA-NTR	0.59	(0.41, 0.84)	1361	H≣H
NEWMOOD-L1-BP	0.61	(0.42,0.88)	928	⊢≣ →:
NEWMOOD-L1-MAN NEWMOOD-L2-MAN	0.44 0.30	(0.34,0.57) (0.16,0.56)	1211 231	
SHIP	0.30	(0.34,0.56)	1980	# →
TRAILS	0.40	(0.28, 0.57)	1200	.
TREND	0.62	(0.51, 0.75)	3151	
Overall summary	0.57	(0.50,0.66)	21135	•
Overall P value			1.4e-15	
				0.0 1.0 2.0 3.0 4.0 5.0 6.0
				Odds Ratio

1a.

Study	OR 95% C.I.	N	
ALSPAC CHDS COGA DeCC EPIC-Norfolk ESPRIT GAN12France GENESIS MARS Molise Muenster NESDA-NTR NEWMOOD-L1-BP NEWMOOD-L1-MAN NEWMOOD-L2-MAN SHIP TRAILS TREND	2.53 (1.25, 5.12) 2.40 (1.18, 4.89) 4.50 (2.20, 9.20) 1.51 (0.70, 3.24) 1.75 (1.02, 2.99) 2.23 (1.31, 3.82) 1.40 (0.18, 10.81) 0.79 (0.47, 1.33) 3.77 (0.72, 19.80) 1.61 (0.91, 2.85) 3.21 (1.60, 6.44) 4.23 (1.41, 12.66) 2.27 (1.12, 4.61) 3.20 (1.94, 5.28) 1.70 (0.37, 7.78) 0.70 (0.36, 1.36) 5.27 (2.67, 10.40) 2.21 (1.46, 3.34)	1209 702 571 444 4023 1301 277 904 299 745 598 1361 928 1211 231 1980 1200 3151	
Overall summary Overall P value	2.16 (1.65, 2.82)	21135 1.7e-08	0.0 1.0 2.0 3.0 4.0 5.0 6.0

Odds Ratio

1b.

Study	OR	95% C.I.	N	
ALSPAC	1.14	(0.96,1.35)	1209	; ∫ =+1
CHDS	1.01	(0.78, 1.31)	702	⊢• i⊣
COGA	1.05	(0.75,1.47)	571	 -
DeCC	0.80	(0.59, 1.09)	444	। = !
EPIC-Norfolk	1.00	(0.89, 1.13)	4023	.
ESPRIT	0.91	(0.75, 1.11)	1301	lii i.
GAN12France	0.53	(0.27, 1.05)	277	 ■
GENESIS	0.97	(0.71,1.34)	904	
MARS Molise	1.18	(0.72,1.96)	299 745	
Muenster	0.89 0.90	(0.65,1.22) (0.70,1.17)	745 598	1 = 1
NESDA-NTR	1.15	(0.88,1.51)	1361	- =
NEWMOOD-L1-BP	0.95	(0.74,1.22)	928	
NEWMOOD-L1-MAN	1.00	(0.83,1.20)	1211	—————————————————————————————————————
NEWMOOD-L2-MAN	1.32	(0.87, 2.00)	231	· · · · · · · · · · · · · · · · · · ·
SHIP	1.03	(0.86, 1.24)	1980	: ■
TRAILS	0.98	(0.76,1.26)	1200	- • →
TREND	0.99	(0.86,1.14)	3151	•
Overall summary	1.00	(0.95,1.05)	21135	•
Overall P value		, ,	0.95	
				0.0 1.0 2.0 3.0 4.0 5.0 6.0
				0.11. 5. "
				Odds Ratio

1c.

Study	OR 95%	C.I. N	
ALSPAC CHDS COGA DeCC EPIC-Norfolk ESPRIT GAN12France GENESIS MARS Molise Muenster NESDA-NTR NEWMOOD-L1-BP NEWMOOD-L1-MAN NEWMOOD-L2-MAN SHIP TRAILS TREND	0.78 (0.40, 1) 0.98 (0.52, 1) 1.14 (0.58, 2) 2.89 (1.28, 6) 0.81 (0.49, 1) 1.01 (0.61, 1) 0.97 (0.21, 4) 0.97 (0.62, 1) 0.38 (0.04, 3) 0.85 (0.51, 1) 1.23 (0.66, 2) 0.83 (0.31, 2) 1.34 (0.73, 2) 1.05 (0.67, 1) 1.91 (0.36, 10) 1.30 (0.72, 2) 0.70 (0.35, 1) 1.24 (0.84, 1)	.83) 702 .23) 571 .51) 444 .32) 4023 .65) 1301 .35) 277 .53) 904 .84) 299 .41) 745 .32) 598 .22) 1361 .47) 928 .66) 1211 .15) 231 .36) 1980 .37) 1200	
Overall summary Overall P value	1.05 (0.91, 1	.21) 21135 0.49	0.0 1.0 2.0 3.0 4.0 5.0 6.0
			Odds Ratio

1d.

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

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

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))

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)

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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

Stress Studies Subjects Covariate OR 95% CD Childhood maltreatment 18 21135 Sex 6.75 (0.50, 0.66) Stress 2.13 Stress 2.16 (1.65, 2.82) Charle Stress 2.87 (1.67, 2.82) (0.50, 0.05) Stress 2.87 (0.51, 1.21) (0.51, 1.21) Stress 2.87 (1.87, 4.41) (0.51, 1.21) Stress 2.87 (1.87, 4.41) (0.51, 1.10) Stress 2.87 (0.51, 1.10) (0.52, 1.10) Broad stress (Other life stress < 5 years prior or childhood maltreatment) 14 13835 Sex 0.53 (0.51, 0.67) Broad stress (Other life stress or childhood maltreatment) 14 13835 Sex 0.60 (0.51, 0.70) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.54, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Gene x stress 100 (0.94, 1.10) Broad stress (Other life stress or childh	w poo	Childhood Maltreatment						
Stress 2113 Stress 216 (165.282)	Depression	Stress	Studies	Subjects	Covariate	OR	95% CI	p-value
Childhood maltreatment 13 13956 Sex 1.05 1.055, 1.051	ime	Childhood maltreatment	18	21135	Sex	0.57	(0.50, 0.66)	1.4E-15
Gene × stress 1.05 (0.95, 1.05) Gene × stress 1.05 (0.91, 1.21) Stress 2.87 (1.87, 4.41) Gene × stress 0.63 (0.51, 0.78) Stress 0.89 (0.51, 0.78) Gene × stress 0.93 (0.76, 1.14) Gene × stress 0.93 (0.76, 1.14) Gene × stress 1.06 (0.95, 1.06) Gene × stress 1.06 (0.95, 1.06) Gene × stress 0.63 (0.51, 0.67) Gene × stress 0.63 (0.51, 0.78) Gene × stress 0.60 (0.53, 0.67) Stress 0.60 (0.53, 0.67) Gene × stress 0.60 (0.54, 1.07) Gene × stress 1.06 (0.94, 1.107) Gene × stress 1.08 (0.94, 1.107)					Stress	2.16	(1.65, 2.82)	1.7E-08
Childhood maltreatment 13 13956 Sex 1.05 (0.91, 1.121)					Gene	1.00	(0.95, 1.05)	0.95
Stress Sex O.51, 0.78					$\text{Gene} \times \text{stress}$	1.05	(0.91, 1.21)	0.49
Stress Stress Stress 1.07 (0.92, 1.10) Broad stress (Other life stress < 5 years prior or childhood maltreatment)	ent	Childhood maltreatment	13	13956	Sex	0.63	(0.51, 0.78)	3.5E-05
Broad stress (Other life stress < 5 years prior or childhood maltreatment) 2 mode stress (Other life stress < 5 years prior or childhood maltreatment) 19					Stress	2.87	(1.87, 4.41)	1.5E-06
Stress Studies Studies Studies Gene × stress 0.93 (0.76, 1.14) Broad stress (Other life stress < 5 years prior or childhood maltreatment)					Gene	1.00	(0.92, 1.10)	0.97
Broad stress (Other life stress < 5 years prior or childhood maltreatment) 19 21938 Sex 0.58 (0.51, 0.67) Broad stress (Other life stress conditions of maltreatment) 14 13835 Sex 0.63 (0.51, 1.06) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.63 (0.51, 1.10) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.54, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.07) Gene x stress 106 0.64, 1.07 Gene x stress 1.06 0.94, 1.07 Gene x stress 108 0.92, 1.27 Gene x stress 1.06 0.94, 1.07 Gene x stress 108 0.92, 1.27 Gene x stress 1.06 0.94, 1.07 Gene x stress 108 0.92, 1.27 Gene x stress 1.06 0.94, 1.07 108 <td< td=""><td></td><td></td><td></td><td></td><td>$\text{Gene} \times \text{stress}$</td><td>0.93</td><td>(0.76, 1.14)</td><td>0.50</td></td<>					$\text{Gene} \times \text{stress}$	0.93	(0.76, 1.14)	0.50
Broad stress (Other life stress < 5 years prior or childhood maltreatment) 19 21938 Sex 0.58 (0.51, 0.67) Broad stress (Other life stress condiduood maltreatment) 14 13835 Sex 0.63 (0.51, 0.67) Broad stress (Other life stress or childhood maltreatment) 14 13835 Sex 0.63 (0.51, 1.08) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.56, 1.11) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.56, 2.56) Broad stress (Other life stress or childhood maltreatment) 17 17015 Stress 2.00 (0.54, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Stress 2.00 (0.54, 1.07)	Stress							
Broad stress (Other life stress < 5 years prior or childhood maltreatment) 19 21938 Sex 0.58 (0.51, 0.67) Broad stress (Other life stress or childhood maltreatment) 14 13835 Sex 0.63 (0.93, 1.20) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.94, 1.17) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.60 (0.54, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16)	ssion	Stress	Studies	Subjects	Covariate	OR	95% CI	p-value
Broad stress (Other life stress of years prior or childhood maltreatment) 14 13835 Sex 1.06 (0.95, 1.06) Broad stress (Other life stress or childhood maltreatment) 21 13835 Sex 0.63 (0.51, 0.78) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.07) Gene × stress 2.06 (1.62, 4.19) Contact of the	ime	Broad stress (Other life stress < 5 years prior or childhood maltreatment)		21938	Sex	0.58	(0.51, 0.67)	2.8E-15
Broad stress (Other life stress of years prior or childhood maltreatment) 14 13835 Sex 1.06 (0.95, 1.06) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.63 (0.51, 0.78) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.44, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Gene × stress 0.66 0.61 0.64, 1.05 0.61 0.64, 0.75					Stress	1.82	(1.39, 2.39)	1.4E-05
Broad stress (Other life stress or childhood maltreatment) 14 13835 Sex 1.06 (0.93, 1.20) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.54, 0.78) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.06 (0.94, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75)					Gene	1.00	(0.95, 1.06)	0.95
Broad stress (Other life stress < 5 years prior or childhood maltreatment) 14 13835 Sex 0.63 (0.51, 0.78) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.10) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75)					Gene × stress	1.06	(0.93, 1.20)	0.40
Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.54, 4.91) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Chene × stress 10 0.94, 1.16 1.06 0.05, 1.17	ent	Broad stress (Other life stress < 5 years prior or childhood maltreatment)		13835	Sex	0.63	(0.51, 0.78)	2.4E-05
Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.90 (0.76, 1.11) Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Agnes × stress 2.60 (1.62, 4.19) Agnes × stress 2.60 (1.62, 4.19) Agnes × stress 0.85 (0.68, 1.07)					Stress	3.19	(2.08, 4.91)	1.2E-07
Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.76, 1.11) Stress 0.60 (0.53, 0.67) Stress 2.00 (1.56, 2.56) Gene × stress 1.00 (0.94, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Stress 2.60 (1.62, 4.19) Gene × stress 1.08 (0.92, 1.27)					Gene	1.01	(0.90, 1.12)	0.91
Broad stress (Other life stress or childhood maltreatment) 21 28252 Sex 0.60 (0.53, 0.67) Stress 200 (1.56, 2.56) Gene × stress 100 (0.94, 1.10) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.94, 1.16) Stress 2.60 (1.62, 4.19) Gene × stress 1.08 (0.92, 1.27)					$\text{Gene} \times \text{stress}$	0.92	(0.76, 1.11)	0.39
Stress 2.00 (1.56, 2.56) Gene 1.00 (0.94, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Stress 2.60 (1.62, 4.19) Gene × stress 1.08 (0.92, 1.27) Gene × stress 0.85 (0.68, 1.07)	ime	Broad stress (Other life stress or childhood maltreatment)	21	28252	Sex	0.60	(0.53, 0.67)	6.5E-17
Gene × stress 1.00 (0.94, 1.07) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Stress 2.60 (1.62, 4.19) Gene × stress 2.60 (1.62, 4.19) Gene × stress 0.85 (0.88, 1.07)					Stress	2.00	(1.56, 2.56)	3.8E-08
Gene × stress 1.05 (0.94, 1.16) Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Stress 2.60 (1.62, 4.19) Gene × stress 1.08 (0.92, 1.27) Gene × stress 0.85 (0.68, 1.07)					Gene	1.00	(0.94, 1.07)	0.92
Broad stress (Other life stress or childhood maltreatment) 17 17015 Sex 0.61 (0.49, 0.75) Stress 2.60 (1.62, 4.19) Gene × stress 0.85 (0.68, 1.07)					$Gene \times stress$	1.05	(0.94, 1.16)	0.38
2.60 (1.62, 4.19) 1.08 (0.92, 1.27) 0.85 (0.68, 1.07)	ent	Broad stress (Other life stress or childhood maltreatment)	17	17015	Sex	0.61	(0.49, 0.75)	5.1E-06
1.08 (0.92, 1.27) 0.85 (0.68, 1.07)					Stress	2.60	(1.62, 4.19)	7.8E-05
0.85 (0.68, 1.07)					Gene	1.08	(0.92, 1.27)	0.35
					Gene × stress	0.85	(0.68, 1.07)	0.17

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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