Molecular Psychiatry (2017) 00, 1–10

www.nature.com/mp

ORIGINAL ARTICLE

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

RC Culverhouse[1], NL Saccone[2], AC Horton[3], Y Ma[3], KJ Anstey[4], T Banaschewski[5], M Burmeister[6,7], S Cohen-Woods[8], B Etain[9,10,11], HL Fisher[12], N Goldman[13], S Guillaume[14,15,16], J Horwood[17], G Juhasz[18,19,20,21], KJ Lester[22], L Mandelli[23], CM Middeldorp[24,25], E Olié[14,15,16], S Villafuerte[6], TM Air[26], R Araya[27], L Bowes[28], R Burns[4], EM Byrne[29], C Coffey[30], WL Coventry[31], KAB Gawronski[32], D Glei[33], A Hatzimanolis[34,35], J-J Hottenga[24,36], I Jaussent[15], C Jawahar[26], C Jennen-Steinmetz[37], JR Kramer[38], M Lajnef[39], K Little[40,41], HM zu Schwabedissen[42], M Nauck[43], E Nederhof[44], P Petschner[19,21,45], WJ Peyrot[46], C Schwahn[47], G Sinnamon[26], D Stacey[26], Y Tian[48], C Toben[26], S Van der Auwera[49], N Wainwright[50], J-C Wang[51], G Willemsen[24,36], IM Anderson[20,52], V Arolt[53], C Åslund[54,55], G Bagdy[19,21,45], BT Baune[26], F Bellivier[9,10,11], DI Boomsma[24,25,36], P Courtet[14,15,16], U Dannlowski[53], EJC de Geus[24,36], JFW Deakin[20,52], S Easteal[56], T Eley[57], DM Fergusson[17], AM Goate[51], X Gonda[19,21,45,58], HJ Grabe[49], C Holzman[48], EO Johnson[59], M Kennedy[60], M Laucht[5], NG Martin[61], MR Munafò[62,63], KW Nilsson[54,55], AJ Oldehinkel[44], CA Olsson[64,65,66], J Ormel[44], C Otte[67], GC Patton[68], BWJH Penninx[46], K Ritchie[15], M Sarchiapone[69], JM Scheid[70], A Serretti[23], JH Smit[46], NC Stefanis[34,35], PG Surtees[50], H Völzke[71], M Weinstein[33], M Whooley[72], JI Nurnberger Jr[73] and LJ Bierut[3]

[1]Department of Medicine and Division of Biostatistics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA; [2]Department of Genetics and Division of Biostatistics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA; [3]Department of Psychiatry, Washington University in St. Louis School of Medicine, St. Louis, MO, USA; [4]Centre for Research on Ageing, Health and Wellbeing, The Australian National University, Canberra, ACT, Australia; [5]Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany; [6]Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA; [7]Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA; [8]School of Psychology, Faculty of Social and Behavioural Sciences, Flinders University, Adelaide, SA, Australia; [9]Sorbonne Paris Cité, Université Paris Diderot, UMR-S 1144, Paris, France; [10]AP-HP, Groupe Saint-Louis-Lariboisière-F. Widal, Paris, France; [11]INSERM, U1144, Paris, France; [12]Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK; [13]Office of Population Research, Princeton University, Princeton, NJ, USA; [14]Université Montpellier, Montpellier, France; [15]INSERM U1061 Neuropsychiatry, Montpellier, France; [16]Department of Emergency Psychiatry and Acute Care, CHU Montpellier, Montpellier, France; [17]Department of Psychological Medicine, University of Otago Christchurch, Christchurch, New Zealand; [18]MTA-SE-NAP B Genetic Brain Imaging Migraine Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary; [19]Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary; [20]Neuroscience and Psychiatry Unit, The University of Manchester, Manchester, UK; [21]NAP-A-SE New Antidepressant Target Research Group, Semmelweis University, Budapest, Hungary; [22]School of Psychology, University of Sussex, Brighton, UK; [23]Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy; [24]Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; [25]Neuroscience Campus Amsterdam, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; [26]Discipline of Psychiatry, University of Adelaide, Adelaide, SA, Australia; [27]Centre for Global Mental Health, London School of Hygiene and Tropical Medicine, London, UK; [28]Department of Experimental Psychology, University of Oxford, Oxford, UK; [29]Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia; [30]Centre for Adolescent Health, Murdoch Childrens Research Institute, Melbourne, VIC, Australia; [31]Discipline of Psychology, University of New England, Armidale, NSW, Australia; [32]Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [33]Center for Population and Health, Georgetown University, Washington, DC, USA; [34]Department of Psychiatry, Eginition Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [35]Neurobiology Research Institute, Theodor-Theohari Cozzika Foundation, Athens, Greece; [36]EMGO+ Institute for Health and Care Research, VU Medical Center Amsterdam, Amsterdam, The Netherlands; [37]Department of Biostatistics, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany; [38]Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA, USA; [39]INSERM U955, Creteil, France; [40]Murdoch Childrens Research Institute, Melbourne, VIC, Australia; [41]Department of Paediatrics and School of Psychological Sciences, University of Melbourne, Melbourne, VIC, Australia; [42]Biopharmacy, Department Pharmaceutical Sciences, University of Basel, Basel, Switzerland; [43]Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany; [44]University of Groningen, University Medical Center Groningen, Interdisciplinary Center Psychopathology and Emotion Regulation, Groningen, The Netherlands; [45]MTA-SE Neuropsychopharmacology and Neurochemistry Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary; [46]Department of Psychiatry, VU University Medical Center & GGZ inGeest, Amsterdam, The Netherlands; [47]Department of Prosthetic Dentistry, Gerostomatology and Dental Materials, University Medicine Greifswald, Greifswald, Germany; [48]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA; [49]Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany; [50]Department of Public Health and Primary Care, School of Clinical Medicine, Cambridge, UK; [51]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [52]Manchester Academic Health Sciences Centre, Manchester, UK; [53]Department of Psychiatry and Psychotherapy, University of Münster, Münster, Germany; [54]Centre for Clinical Research, Uppsala University, Uppsala, Sweden; [55]Västmanland County Hospital Västerås, Västerås, Sweden; [56]John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia; [57]King's College London, Institute of Psychiatry, Psychology & Neuroscience, London, UK; [58]Department of Psychiatry and Psychotherapy, Kutvolgyi Clinical Center, Semmelweis University, Budapest, Hungary; [59]Fellow Program and Behavioral Health and Criminal Justice Division, RTI International, Research Triangle Park, NC, USA; [60]Department of Pathology, University of Otago Christchurch, Christchurch, New Zealand; [61]Genetic Epidemiology, QIMR Berghofer, Brisbane, QLD, Australia; [62]MRC Integrative Epidemiology Unit at the University of Bristol, Bristol, UK; [63]UK Centre for Tobacco and Alcohol Studies, School of Experimental Psychology, University of Bristol, Bristol, UK; [64]Deakin University Geelong, Centre for Social and Early Emotional Development, School of Psychology, Faculty of Health, Burwood, VIC, Australia; [65]Department of Paediatrics and School of Psychological Sciences, University of Melbourne, Melbourne, VIC, Australia; [66]Centre for Adolescent Health, Murdoch Childrens Research Institute, Melbourne, VIC, Australia; [67]Charité Universitätsmedizin Berlin, Klinik für Psychiatrie und Psychotherapie Campus Benjamin Franklin, Berlin, Germany; [68]Department of Paediatrics, Murdoch Childrens Research Institute, University of Melbourne, Melbourne, VIC, Australia; [69]Department of Health Sciences, University of Molise, Campobasso, Italy; [70]Department of Psychiatry, Michigan State University, East Lansing, MI, USA; [71]Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany; [72]Veterans Affairs Health Care System and University of California, San Francisco, CA, USA and [73]Institute of Psychiatric Research, Departments of Psychiatry and Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA. Correspondence: Dr R Culverhouse, Internal Medicine, Washington University in Saint Louis School of Medicine, Campus Box 8005. 4523 Clayton Avenue, St. Louis, MO 63110, USA.
E-mail: rculverh@wustl.edu
Received 22 August 2016; revised 23 January 2017; accepted 2 February 2017

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 data sets containing 38 802 European ancestry subjects genotyped for 5-HTTLPR and assessed for depression and childhood maltreatment or other stressful life events, and meta-analysed 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) 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 generalisable, but must be of modest effect size and only observable in limited situations.

Molecular Psychiatry advance online publication, 4 April 2017; doi:10.1038/mp.2017.44

INTRODUCTION

Depression negatively impacts health more than any other chronic disease[1] and is a leading cause of total disease burden worldwide.[2] Both genetic and environmental factors influence depression;[3] research on the aetiology of depression suggests substantial heritability of 40–50%.[3–8] Only recently have genome-wide association studies begun to identify and replicate specific loci associated with depression.[9–11] The findings from these studies suggest that (1) the effects of individual single-nucleotide polymorphisms on major depressive disorder are small in magnitude (requiring large sample sizes to detect) and (2) candidate genes generally do not show evidence of association in either genome-wide association studies or subsequent large-scale meta-analyses.[12] Gene-environment interactions (G × E) (for example, 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.[13,14]

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.[15] *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 with the long (L) allele.[16,17] 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 among those with two copies of the S allele compared with 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 have 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.[18] Furthermore, many candidate gene main-effect association reports appear to be false positives.[19,20] As Duncan and Keller[21] 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,[22–25] with various reasons proposed for the differences.[21,24,26–28] Munafò *et al.*[23] performed a literature-based meta-analysis, finding that only five 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.*[22] obtained individual level data from 10 previously published studies ($N = 14\,250$) that met inclusion criteria and analysed 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.*[24] (56 studies, $N = 40\,749$) and Sharpley *et al.*[25] (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.* criticised 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 criticised 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.[21] One key issue contributing to disputes over the appropriateness of the prior reports and meta-analyses is the heterogeneity of the studies.[21] 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 have 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.[29] Our collaborative meta-analysis strategy, wherein the consortium worked to harmonise

phenotypes across studies, to prioritise specific analyses *a priori*, and to apply identical *de novo* statistical analyses across all participating studies, provided a balance between maximising sample size while minimising 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.

MATERIALS AND METHODS

Coordinated meta-analysis process

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. Supplementary Table S1 shows the data sets contributing to this meta-analysis and how they relate to the Risch meta-analysis[22] based on primary data and the three literature-based meta-analyses of Munafò, Karg and Sharpley.[23–25] 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]

Development of the protocol. The consortium developed an analysis protocol that focused on data harmonisation and analysis prioritisation. The decision was made to analyse 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.[15]

All analyses were stratified by genetic ancestry. An outline of the primary analyses can be found in Supplementary 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.

Analysis script. The coordinating center at Washington University in St Louis developed data coding instructions (Supplementary 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 meta-analysis.

Quality control assessments

Data coding: To ensure high quality data, the analysis team at Washington University examined the submitted results for unusual values (for example, 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.

Meta-analysis

Meta-analyses of both the primary and secondary models were performed using the R packages rmeta[63] 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 Supplementary Table S4).

Models analysed

In keeping with the original report,[15] 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 × 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 (for example, 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 (for example, 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 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 harmonisation 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-analysed owing to small sample size. Supplementary Table S5 provides key demographic information about the data included in the meta-analyses (for example, N, S allele frequency, frequencies of the key phenotypes). For each of the data sets in Supplementary Table S5, Supplementary 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. Supplementary Table S7 provides information about

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

Depression	*Stress*	*Studies*	*Subjects*	*Covariate*	*OR*	*95% CI*	P-*value*
Childhood maltreatment							
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 × 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 × stress	0.93	(0.76, 1.14)	0.50
Broad stress							
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 × 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 × 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 × 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 × 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 Supplementary 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.

additional data sets 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 Supplementary 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.[15]

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 with 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 (5 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*.[15] 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 summarised in Table 1 are given in Supplementary Figures S1 through S5. Forest plots for the interaction terms for the remaining primary and secondary analyses are given in Supplementary Figures S6 to S14.

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

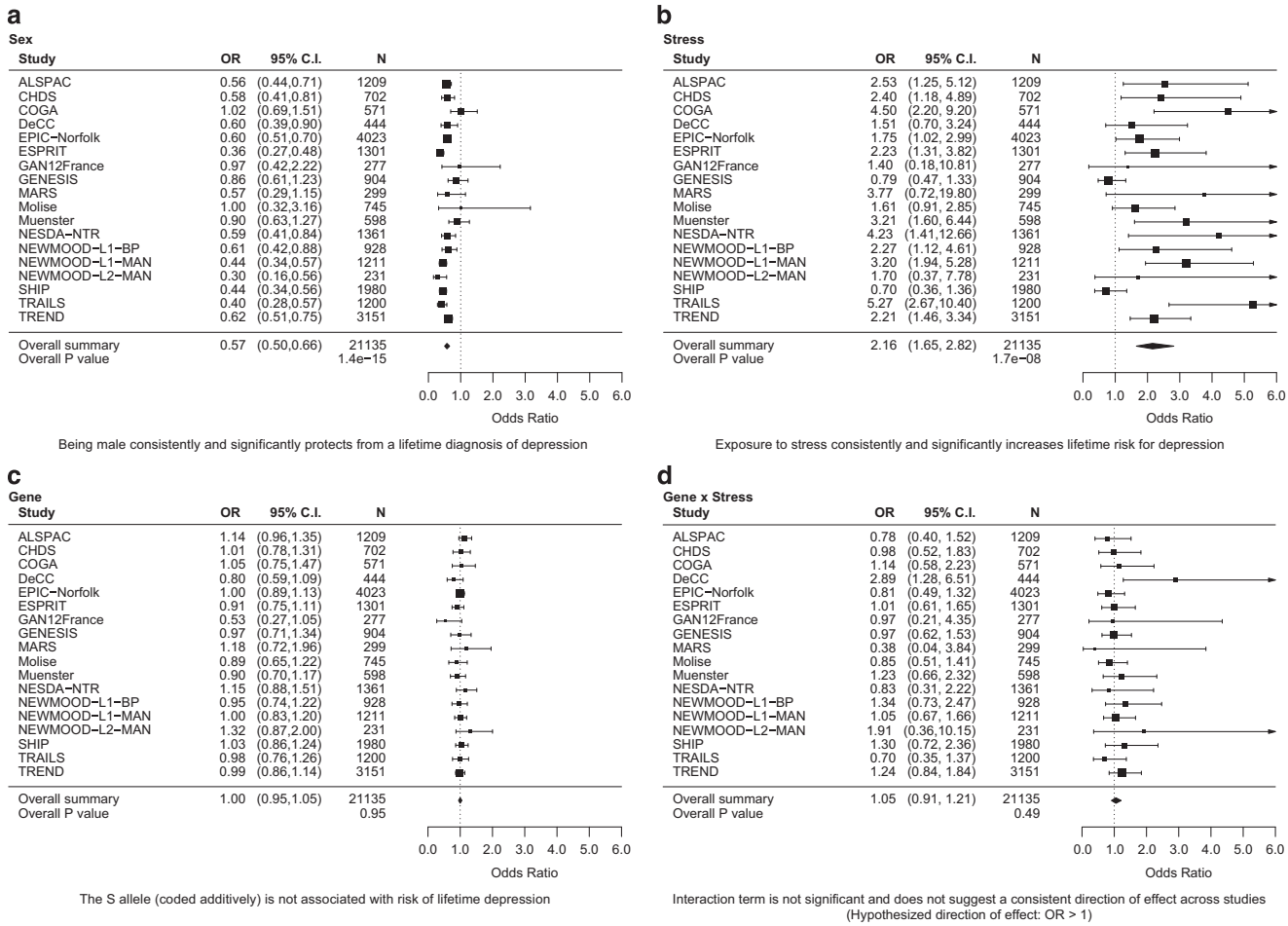

Figure 1. Forest plots for the four key factors for the first model listed in Table 1. (**a**) Sex: odds ratio (OR) = 0.57, $p = 1.4e\text{-}15$; (**b**) stress: OR = 2.16, $p = 1.7e\text{-}8$; (**c**) gene: OR = 1.00, $p = 0.95$; (**d**) gene × Stress: OR = 1.05, $p = 0.49$. This analysis examined the outcome lifetime depression diagnosis in subjects of all ages based on exposure to childhood maltreatment as the stressor. Sex and stress display significant and consistent effects across the studies. The main effect of 5-HTTLPR and the interaction between 5-HTTLPR and stress are not significant. 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)).

< 0.05 before correction for multiple tests) in the hypothesised direction (Supplementary 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 (Supplementary Tables S10b, S9), and the matching analysis with subjects of all ages has the point estimates of effect in the opposite direction (Supplementary 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 hypothesised 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 Diagnostic and Statistical Manual (DSM) or International Statistical Classification of Diseases and Related Health Problems (ICD) criteria (Supplementary Table S13). To determine whether the interaction might be predominantly expressed in only one sex, we performed meta-analyses stratified by sex (Supplementary Table S14). We examined alternative coding of the genetic effect (dominant, recessive, haplotype) (Supplementary 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 (Supplementary 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 (Supplementary 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 hypothesised direction (Supplementary 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. Supplementary 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 generalisable interaction effect reported in Caspi *et al*.[15]

DISCUSSION

A hallmark of science is the ability of results to be replicated, a criterion that has been increasingly recognised in biological and psychological research.[66] The original 2003 report of an interaction between 5-HTTLPR genotype and stress exposure on depression[15] has remained controversial owing to inconsistent results from replication efforts. Although some researchers have claimed a replication of the hypothesised interaction based on different stressors, different measures of depression or different genetic models,[24,25] other attempts to replicate the finding have been negative.[22,23,67] The goal of our study was to rigorously explore the extent to which the original report could be replicated and generalised using a structured collaborative meta-analysis.

This is the largest study to date to use consistent statistical analyses across all samples to examine the hypothesised 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 $< 10^{-60}$ were seen in our most inclusive primary analyses (Supplementary 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 hypothesised 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[15] 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 criticised.[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 recognise 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 hypothesised direction were based on lifetime depression outcomes. Finally, based on parameter estimates provided in the supplement to their seminal paper,[15] 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*.[15] 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 analysed using the same statistical model for all studies.

Although our consortium tested many high-priority combinations of factors (see Supplementary 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[28,72] 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 hypothesised interaction.

Our novel contribution is to apply a consistent methodology across the participating studies to query a broad range of questions about the hypothesised interaction. Although these studies remain varied in their original design, our unified approach to phenotype harmonisation 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,[15] with the re-examination of the topic using primary data by Risch *et al.*,[22] and with prior meta-analyses.[22–25] 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 generalisable 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.

CONFLICT OF INTEREST

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 3 years he has received compensations for his contributions as member of advisory boards and for presentations for the following companies: AstraZeneca, 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, 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 Us 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. E Olié has received a research grant from AstraZeneca and speaking fees from Otsuka, AstraZeneca, Lundbeck. She is involved in clinical trail conducted by 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 Glei, 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, 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.

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. LB receives funding as an Early Career Research Fellow from the Leverhulme Trust. 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. We 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. COGEND: 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 Woods 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). NEW-MOOD: 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-Königska 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 (Integrament) 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 Individualised 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 centres (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:

We 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. 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: We 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 11 different centres: University of Connecticut (V Hesselbrock); Indiana University (HJ 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 (JP 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 Oreland, 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 Professor 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 Professor 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.

REFERENCES

1 Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. *Lancet* 2007; **370**: 851–858.
2 Ustun TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. *Br J Psychiatry* 2004; **184**: 386–392.
3 Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* 2000; **157**: 1552–1562.
4 Bierut LJ, Heath AC, Bucholz KK, Dinwiddie SH, Madden PA, Statham DJ *et al.* Major depressive disorder in a community-based twin sample: are there different genetic and environmental contributions for men and women? *Arch Gen Psychiatry* 1999; **56**: 557–563.
5 Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. The lifetime history of major depression in women. Reliability of diagnosis and heritability. *Arch Gen Psychiatry* 1993; **50**: 863–870.
6 Kendler KS, Gardner CO, Prescott CA. Are there sex differences in the reliability of a lifetime history of major depression and its predictors? *Psychol Med* 2001; **31**: 617–625.
7 McGuffin P, Katz R, Rutherford J. Nature, nurture and depression: a twin study. *Psychol Med* 1991; **21**: 329–335.
8 McGuffin P, Katz R, Watkins S, Rutherford J. A hospital-based twin register of the heritability of DSM-IV unipolar depression. *Arch Gen Psychiatry* 1996; **53**: 129–136.
9 Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G *et al.* A mega-analysis of genome-wide association studies for major depressive disorder. *Mol Psychiatry* 2013; **18**: 497–511.
10 Flint J, Kendler KS. The genetics of major depression. *Neuron* 2014; **81**: 484–503.
11 Hyde CL, Nagle MW, Tian C, Chen X, Paciga SA, Wendland JR *et al.* Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. *Nat Genet* 2016; **48**: 1031–1036.
12 Dunn EC, Brown RC, Dai Y, Rosand J, Nugent NR, Amstadter AB *et al.* Genetic determinants of depression: recent findings and future directions. *Harv Rev Psychiatry* 2015; **23**: 1–18.

13 Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC *et al.* Stressful life events, genetic liability, and onset of an episode of major depression in women. *Am J Psychiatry* 1995; **152**: 833–842.
14 Cohen-Woods S, Craig IW, McGuffin P. The current state of play on the molecular genetics of depression. *Psychol Med* 2013; **43**: 673–687.
15 Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; **301**: 386–389.
16 Lesch KP, Greenberg MD, Higley JD, Bennett A, Murphy DL. Serotonin transporter, personality, and behavior: toward a disection of gene-gene and gene-environment interaction. In: Benjamin J, Ebstein RP, Belmaker RH (eds). *Molecular Genetics and the Human Personality. American Psychiatric Association*. APA: Washington, DC, 2002, pp 109–136.
17 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1531.
18 McClelland GH, Judd CM. Statistical difficulties of detecting interactions and moderator effects. *Psychol Bull* 1993; **114**: 376–390.
19 Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D *et al.* Poor replication of candidate genes for major depressive disorder using genome-wide association data. *Mol Psychiatry* 2011; **16**: 516–532.
20 Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL *et al.* A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet* 2009; **5**: e1000373.
21 Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *Am J Psychiatry* 2011; **168**: 1041–1049.
22 Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J *et al.* Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA* 2009; **301**: 2462–2471.
23 Munafo MR, Durrant C, Lewis G, Flint J. Gene X environment interactions at the serotonin transporter locus. *Biol Psychiatry* 2009; **65**: 211–219.
24 Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Arch Gen Psychiatry* 2011; **68**: 444–454.
25 Sharpley CF, Palanisamy SK, Glyde NS, Dillingham PW, Agnew LL. An update on the interaction between the serotonin transporter promoter variant (5-HTTLPR), stress and depression, plus an exploration of non-confirming findings. *Behav Brain Res* 2014; **273**: 89–105.
26 Brown GW, Harris TO. Depression and the serotonin transporter 5-HTTLPR polymorphism: a review and a hypothesis concerning gene-environment interaction. *J Affect Disord* 2008; **111**: 1–12.
27 Munafo MR, Flint J. Replication and heterogeneity in gene x environment interaction studies. *Int J Neuropsychopharmacol* 2009; **12**: 727–729.
28 Uher R, Caspi A, Houts R, Sugden K, Williams B, Poulton R *et al.* Serotonin transporter gene moderates childhood maltreatment's effects on persistent but not single-episode depression: replications and implications for resolving inconsistent results. *J Affect Disord* 2011; **135**: 56–65.
29 Culverhouse RC, Bowes L, Breslau N, Nurnberger JI Jr., Burmeister M, Fergusson DM *et al.* Protocol for a collaborative meta-analysis of 5-HTTLPR, stress, and depression. *BMC Psychiatry* 2013; **13**: 304.
30 Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J *et al.* Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013; **42**: 111–127.
31 Stefanis NC, Mandelli L, Hatzimanolis A, Zaninotto L, Smyrnis N, Avramopoulos D *et al.* Serotonin transporter gene variants and prediction of stress-induced risk for psychological distress. *Genes Brain Behav* 2011; **10**: 536–541.
32 Edwards BHM, Letcher P, Little K, Macdonald J, Oberklaid F, O'Connor M, *et al. The Australian Temperament Project: The first 30 years*. Australian Institute of Family Studies: Commonwealth of Australia, 2013.
33 Fergusson DM, Horwood LJ. The Christchurch Health and Development Study. In: Joyce P, Nicholls G, Thomas K, Wilkinson T (eds). *The Christchurch Experience: 40 Years of Research and Teaching*. University of Otago: Christchurch, 2013, pp 79–87.
34 Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF *et al.* Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 2007; **16**: 24–35.
35 Cohen-Woods S, Gaysina D, Craddock N, Farmer A, Gray J, Gunasinghe C *et al.* Depression Case Control (DeCC) Study fails to support involvement of the muscarinic acetylcholine receptor M2 (CHRM2) gene in recurrent major depressive disorder. *Hum Mol Genet* 2009; **18**: 1504–1509.
36 Surtees PG, Wainwright NW, Willis-Owen SA, Luben R, Day NE, Flint J. Social adversity, the serotonin transporter (5-HTTLPR) polymorphism and major depressive disorder. *Biol Psychiatry* 2006; **59**: 224–229.
37 Ritchie K, Artero S, Beluche I, Ancelin ML, Mann A, Dupuy AM *et al.* Prevalence of DSM-IV psychiatric disorder in the French elderly population. *Br J Psychiatry* 2004; **184**: 147–152.
38 Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P *et al.* Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol Psychiatry* 2004; **9**: 908–915.
39 Etain B, Lajnef M, Henrion A, Dargel AA, Stertz L, Kapczinski F *et al.* Interaction between SLC6A4 promoter variants and childhood trauma on the age at onset of bipolar disorders. *Sci Rep* 2015; **5**: 16301.
40 Penas-Lledo E, Guillaume S, Naranjo ME, Delgado A, Jaussent I, Blasco-Fontecilla H *et al.* A combined high CYP2D6-CYP2C19 metabolic capacity is associated with the severity of suicide attempt as measured by objective circumstances. *Pharmacogenomics J* 2015; **15**: 172–176.
41 Otte C, McCaffery J, Ali S, Whooley MA. Association of a serotonin transporter polymorphism (5-HTTLPR) with depression, perceived stress, and norepinephrine in patients with coronary disease: the Heart and Soul Study. *Am J Psychiatry* 2007; **164**: 1379–1384.
42 Laucht M, Treutlein J, Blomeyer D, Buchmann AF, Schmid B, Becker K *et al.* Interaction between the 5-HTTLPR serotonin transporter polymorphism and environmental adversity for mood and anxiety psychopathology: evidence from a high-risk community sample of young adults. *Int J Neuropsychoph* 2009; **12**: 737–747.
43 Zucker RA, Ellis DA, Fitzgerald HE, Bingham CR, Sanford K. Other evidence for at least two alcoholisms .2. Life course variation in antisociality and heterogeneity of alcoholic outcome. *Dev Psychopathol* 1996; **8**: 831–848.
44 Juhasz G, Gonda X, Hullam G, Eszlari N, Kovacs D, Lazary J *et al.* Variability in the effect of 5-HTTLPR on depression in a large European population: the role of age, symptom profile, type and intensity of life stressors. *PLoS ONE* 2015; **10**: e0116316.
45 Anstey KJ, Christensen H, Butterworth P, Easteal S, Mackinnon A, Jacomb T *et al.* Cohort profile: the PATH through life project. *Int J Epidemiol* 2012; **41**: 951–960.
46 Scheid JM, Holzman CB, Jones N, Friderici KH, Nummy KA, Symonds LL *et al.* Depressive symptoms in mid-pregnancy, lifetime stressors and the 5-HTTLPR genotype. *Genes Brain Behav* 2007; **6**: 453–464.
47 Coventry WL, James MR, Eaves LJ, Gordon SD, Gillespie NA, Ryan L *et al.* Do 5HTTLPR and stress interact in risk for depression and suicidality? Item response analyses of a large sample. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 757–765.
48 Sjoberg RL, Nilsson KW, Nordquist N, Ohrvik J, Leppert J, Lindstrom L *et al.* Development of depression: sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. *Int J Neuropsychopharmacol* 2006; **9**: 443–449.
49 Aslund C, Leppert J, Comasco E, Nordquist N, Oreland L, Nilsson KW. Impact of the interaction between the 5HTTLPR polymorphism and maltreatment on adolescent depression. A population-based study. *Behav Genet* 2009; **39**: 524–531.
50 Goldman N, Glei DA, Lin YH, Weinstein M. The serotonin transporter polymorphism (5-HTTLPR): allelic variation and links with depressive symptoms. *Depress Anxiety* 2010; **27**: 260–269.
51 Volzke H, Alte D, Schmidt CO, Radke D, Lorbeer R, Friedrich N *et al.* Cohort profile: the study of health in Pomerania. *Int J Epidemiol* 2011; **40**: 294–307.
52 Oldehinkel AJ, Rosmalen JG, Buitelaar JK, Hoek HW, Ormel J, Raven D *et al.* Cohort profile update: the TRacking Adolescents' Individual Lives Survey (TRAILS). *Int J Epidemiol* 2015; **44**: 76–76n.
53 Mandelli L, Serretti A, Marino E, Pirovano A, Calati R, Colombo C. Interaction between serotonin transporter gene, catechol-O-methyltransferase gene and stressful life events in mood disorders. *Int J Neuropsychopharmacol* 2007; **10**: 437–447.
54 Carli V, Mandelli L, Zaninotto L, Roy A, Recchia L, Stoppia L *et al.* A protective genetic variant for adverse environments? The role of childhood traumas and serotonin transporter gene on resilience and depressive severity in a high-risk population. *Eur Psychiatry* 2011; **26**: 471–478.
55 Olsson CA, Foley DL, Parkinson-Bates M, Byrnes G, McKenzie M, Patton GC *et al.* Prospects for epigenetic research within cohort studies of psychological disorder: a pilot investigation of a peripheral cell marker of epigenetic risk for depression. *Biol Psychol* 2010; **83**: 159–165.
56 Baune BT, Air TM. Clinical, functional and biological correlates of cognitive dimensions in major depressive disorder - rationale, design, and characteristics of the Cognitive Function and Mood Study (CoFaM-Study). *Front Psychiatry* 2016; **7**: 150, in press.
57 Peyrot WJ, Middeldorp CM, Jansen R, Smit JH, de Geus EJ, Hottenga JJ *et al.* Strong effects of environmental factors on prevalence and course of major depressive disorder are not moderated by 5-HTTLPR polymorphisms in a large Dutch sample. *J Affect Disord* 2013; **146**: 91–99.

58 Reich T. A genomic survey of alcohol dependence and related phenotypes: results from the Collaborative Study on the Genetics of Alcoholism (COGA). *Alcohol Clin Exp Res* 1996; **20**: 133A–137A.
59 Krug EG, Mercy JA, Dahlberg LL, Zwi AB. The world report on violence and health. *Lancet* 2002; **360**: 1083–1088.
60 Hovens JG, Giltay EJ, van Hemert AM, Penninx BW. Childhood maltreatment and the course of depressive and anxiety disorders: the contribution of personality characteristics. *Depress Anxiety* 2016; **33**: 27–34.
61 Fergusson DM, Boden JM, Horwood LJ. Exposure to childhood sexual and physical abuse and adjustment in early adulthood. *Child Abuse Negl* 2008; **32**: 607–619.
62 R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria, 2008.
63 Lumley T. rmeta: Meta-analysis. *R package version* 2.16. 2012.
64 Veichtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Soft* 2010; **36**: 48.
65 SAS/STAT. 9.1 edn. SAS Institute Inc.: Cary, NC, USA, 2002-2003.
66 Open Science C. PSYCHOLOGY. Estimating the reproducibility of psychological science. *Science* 2015; **349**: aac4716.
67 Fergusson DM, Horwood LJ, Miller AL, Kennedy MA. Life stress, 5-HTTLPR and mental disorder: findings from a 30-year longitudinal study. *Br J Psychiatry* 2011; **198**: 129–135.
68 Moffitt TE, Caspi A. Bias in a protocol for a meta-analysis of 5-HTTLPR, stress, and depression. *BMC Psychiatry* 2014; **14**: 179.
69 Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES *et al.* Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 2013; **14**: 365–376.
70 Ioannidis JP. Why most discovered true associations are inflated. *Epidemiology* 2008; **19**: 640–648.
71 Gonda X, Eszlari N, Kovacs D, Anderson IM, Deakin JF, Juhasz G *et al.* Financial difficulties but not other types of recent negative life events show strong interactions with 5-HTTLPR genotype in the development of depressive symptoms. *Transl Psychiatry* 2016; **6**: e798.
72 Brown GW, Ban M, Craig TK, Harris TO, Herbert J, Uher R. Serotonin transporter length polymorphism, childhood maltreatment, and chronic depression: a specific gene-environment interaction. *Depress Anxiety* 2013; **30**: 5–13.
73 Mandelli L, Petrelli C, Serretti A. The role of specific early trauma in adult depression: a meta-analysis of published literature. Childhood trauma and adult depression. *Eur Psychiatry* 2015; **30**: 665–680.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)