Therapygenetic effects of *5-HTT*LPR on cognitive-behavioral therapy in anxiety disorders:

A meta-analysis

Therapygenetics of *5-HTT*LPR in anxiety disorders

Miriam A. Schiele, PhD [1, *], Andreas Reif, MD [2], Jiaxi Lin, PhD [1], Georg W. Alpers, PhD [3], Evelyn Andersson, MSc [4], Gerhard Andersson, PhD [5], Volker Arolt, MD [6], Jan Bergström, PhD [7], Per Carlbring, PhD [7], Thalia C. Eley, MD [8], Gabriel Esquivel, MD, PhD [9], Tomas Furmark, PhD [10], Alexander L. Gerlach, PhD [11], Alfons Hamm, PhD [12], Sylvia Helbig-Lang, PhD [13], Jennifer L. Hudson, PhD [14], Thomas Lang, PhD[15,16], Kathryn J. Lester, DPhil [17], Nils Lindefors [4,18], Tina B. Lonsdorf, PhD [19], Paul Pauli, PhD [20], Jan Richter, PhD [12], Winfried Rief, PhD [21], Susanna Roberts, PhD [8], Christian Rück, MD [4,18], Koen R. J. Schruers, MD, PhD [9], Christiane Thiel, PhD [1], Hans-Ulrich Wittchen, PhD [22], Katharina Domschke, MD, PhD [1,23], Heike Weber, PhD [2,24 #], Ulrike Lueken, PhD [24,25 #]

[#] Both authors contributed equally to this work and should therefore both be considered last author.

[1] Department of Psychiatry and Psychotherapy, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[2] Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, University Hospital Frankfurt, Frankfurt am Main, Germany

[3] Department of Psychology, School of Social Sciences, University of Mannheim, Mannheim, Germany

[4] Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

[5] Department of Behavioural Sciences and Learning, Division of Psychology, Linköping University, Linköping, Sweden

[6] Institute of Translational Psychiatry, Department of Psychiatry and Psychotherapy, University of Muenster, Muenster, Germany

[7] Department of Psychology, Stockholm University, Stockholm, Sweden

[8] King's College London, Institute of Psychiatry, Psychology and Neuroscience, Social, Genetic and Developmental Psychiatry (SGDP) Centre, London, UK.

[9] School for Mental Health and Neuroscience, Maastricht University, The Netherlands and Mondriaan Mental Health Center, Maastricht, The Netherlands

[10] Department of Psychology, Uppsala University, Uppsala, Sweden

[11] Department of Clinical Psychology and Psychotherapy, University of Cologne, Cologne, Germany

[12] Department of Psychology, University of Greifswald, Greifswald, Germany

[13] Department of Clinical Psychology and Psychotherapy, University of Hamburg, Hamburg, Germany

[14] Department of Psychology, Centre for Emotional Health, Macquarie University, Sydney, NSW, Australia

[15] Christoph-Dornier-Foundation for Clinical Psychology, Bremen, Germany

[16] Department of Psychology and Methods, Jacobs University Bremen, Germany

[17] School of Psychology, University of Sussex, Brighton, UK

[18] Stockholm Health Care Services, Region Stockholm, Sweden

[19] Institute for Systems Neuroscience, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[20] Department of Psychology (Biological Psychology, Clinical Psychology, and Psychotherapy), and Center of Mental Health, Julius-Maximilians-Universität Würzburg, Würzburg, Germany

[21] Division of Clinical Psychology and Psychotherapy, Department of Psychology, Philipps University Marburg, Marburg, Germany

[22] Institute of Clinical Psychology and Psychotherapy, Technische Universität Dresden, Dresden, Germany

[23] Center for Basics in NeuroModulation, Faculty of Medicine, University of Freiburg, Germany

[24] Center of Mental Health, Department of Psychiatry, Psychosomatics and Psychotherapy, University Hospital of Würzburg, Germany

[25] Department of Psychology, Humboldt-Universität zu Berlin, Berlin, Germany

*** CORRESPONDING AUTHOR**

Dr. Miriam Schiele, Department of Psychiatry and Psychotherapy, University of Freiburg, Hauptstrasse 5, D-79106 Freiburg, Germany, Tel: +49-761-27069690, Fax: +49-761-27066693, Email: miriam.schiele@uniklinik-freiburg.de

ABSTRACT

There is a recurring debate on the role of the serotonin transporter gene linked polymorphic region (*5-HTT*LPR) in the moderation of response to cognitive behavioral therapy (CBT) in anxiety disorders. Results, however, are still inconclusive. We here aim to perform a meta-analysis on the role of *5-HTT*LPR in the moderation of CBT outcome in anxiety disorders. We investigated both categorical (symptom reduction of at least 50%) and dimensional outcomes from baseline to post-treatment and follow-up. Original data were obtained from ten independent samples (including three unpublished samples) with a total of 2,195 patients with primary anxiety disorder. No significant effects of *5-HTT*LPR genotype on categorical or dimensional outcomes at post and follow-up were detected. We conclude that current evidence does not support the hypothesis of *5-HTT*LPR as a moderator of treatment outcome for CBT in anxiety disorders. Future research should address whether other factors such as long-term changes or epigenetic processes may explain further variance in these complex gene-environment interactions and molecular-genetic pathways that may confer behavioral change following psychotherapy.

KEYWORDS serotonin transporter gene, therapygenetics, treatment response, therapy outcome, CBT, panic disorder

1. INTRODUCTION

Anxiety disorders constitute the largest group of mental disorders with 12-month prevalence rates between 14.0% (EU; Wittchen et al., 2011) and 22.2% (USA; Kessler et al., 2012) and are one of the leading causes of disability worldwide. They are a major precursor for depressive disorders, present with high chronicity and confer a substantial individual and socioeconomic burden, with total costs attributed to anxiety disorders being estimated at 74 billion Euros per year in 2010 (Gustavsson et al., 2011). Anxiety disorders are considered to be complex-genetic disorders, with heritability estimates between 32% and 67% (Hettema et al., 2001; Kendler et al., 1999), comprising the interplay of multiple vulnerability genes of small individual effect.

For the treatment of anxiety disorders, effective pharmacological and psychotherapeutic options are available, however, over one third to 50% of patients with anxiety disorders do not respond to the initial mode of treatment in a clinically significant way (Bystritsky, 2006; Loerinc et al., 2015). In recent years, in an effort to determine predictive markers of successful response to a particular form of treatment and to enable progress towards a "precision medicine" approach (cf. Domschke et al., 2015), a growing body of research has begun to address genetic factors that may be involved in moderating treatment outcome in anxiety disorders, both in relation to pharmacological treatment – thus termed "pharmacogenetics" – and, to a lesser extent, psychotherapies like cognitive behavioral therapy (CBT), correspondingly coined "therapygenetics" (see Eley, 2014; Eley et al., 2012).

Among those studies, efforts have predominately focused on candidate genes related to serotonergic function (see Lueken et al., 2016), particularly on a 44-base pair functional insertion/deletion polymorphism in the promoter region of the serotonin transporter (*5-HTT*; *SLC6A4*) gene – the serotonin transporter gene linked polymorphic region (*5-HTT*LPR). The *5-HTT*LPR consists of a 14 repeat short allele (S) conferring lower *5-HTT* expression levels as compared to the 16 repeat long allele (L), which in turn confers high gene expression (Lesch et al., 1996). A single nucleotide polymorphism has been identified within *5-HTT*LPR

(rs25531 A>G) additionally influencing gene expression in L allele carriers, with the G allele (L_G) rendering it functionally equivalent to the S allele, while presence of the A allele (L_A) leads to increased *5-HTT* expression (Hu et al., 2006; Wendland et al., 2006).

A variety of studies have addressed the potential involvement of the *5-HTT*LPR genotype in the pathogenesis of anxiety disorders *per se* (e.g. Deckert et al., 1997; Hamilton et al., 1999; Maron et al., 2005; Strug et al., 2010) (for meta-analysis see Blaya et al., 2007), as well as with regard to intermediate anxiety phenotypes (e.g. Domschke et al., 2006; Klauke et al., 2011; Klumpers et al., 2012; Lueken et al., 2015; Maron et al., 2004; Schruers et al., 2011), in response to first line pharmacological treatment (Lohoff et al., 2013; Perna et al., 2005; Stein et al., 2006) (for meta-analysis see Porcelli et al., 2012) and in relation to fear extinction as a laboratory analogue of exposure therapy (Agren et al., 2012; Lonsdorf et al., 2009). Results have, however, been equivocal, with either no association, association with the S allele or, conversely, the L allele being reported. Similarly, studies investigating the influence of *5-HTT*LPR on CBT outcome in anxiety disorders have yielded contradictory results reporting either no association (Andersson et al., 2013; Lester et al., 2016; Lonsdorf et al., 2010; Lueken et al., 2015) or a more favorable response conferred by the S allele (Eley et al., 2012; Knuts et al., 2014). These inconsistencies may indicate that the assumed effects are either very small, resulting in the need of larger sample sizes with adequate statistical power. In addition, publication bias, sample heterogeneity, or bi-allelic (*5-HTT*LPR) and tri-allelic approaches (*5-HTT*LPR/rs25531) may account for equivocal finings. Therefore, the aim of the present study was to conduct a meta-analysis of data available of therapygenetic studies in anxiety disorders, both published and unpublished, on the role of *5-HTT*LPR genotype in the moderation of CBT outcome in an attempt to reconcile previous conflicting findings. In particular, we investigated whether this polymorphism exerts effects on categorical vs. dimensionally defined outcomes. Further, if available, we included information regarding comorbid psychotropic medication and rs25531 genotype.

2. EXPERIMENTAL PROCEDURES

2.1 Protocol

This meta-analysis was conducted in accordance with PRISMA guidelines (Liberati et al., 2009). Details of the protocol were registered on the International Prospective Register of Systematic Reviews (PROSPERO; CRD42017070731) and can be accessed at http://www.crd.york.ac.uk/PROSPERO.

2.2 Search strategy and inclusion criteria

Relevant articles published until June 2020 were identified by searching PubMed, CENTRAL, Web of Science, and PsycINFO by title and abstract. A detailed overview of the search terms applied is given in the supplement (Supplementary Table S1). Additional studies were identified manually by searching reference lists of selected articles and pertinent review articles or author contact. Inclusion criteria were defined as (1) peer-reviewed original research published in English or German, (2) primary diagnosis of specific phobia, social anxiety disorder, agoraphobia, panic disorder, or generalized anxiety disorder according to standardized diagnostic criteria (DSM or ICD)[a], (3) documented CBT treatment, (4) pre- and post-treatment assessment time points, and (5) assessment of *5-HTT*LPR (with or without rs25531). Comorbid mental disorders were allowed unless constituting the clinical lead diagnosis. If available, follow-up data (minimum of 6 months post-treatment) were requested. Given the early age of onset of anxiety disorders (Lijster et al., 2017), no limit regarding age range was specified. All studies complied with the Declaration of Helsinki and were approved by the respective local ethical committees. Informed consent/assent was obtained from all participants.

2.3 Data extraction and study characteristics

Results of the literature search are given in Figure 1. Data extraction was performed independently by three researchers (MAS, JL and UL). Discrepancies were resolved by consensus. The initial search yielded 1,288 hits. After removing duplicate results, a total of 781 publications were screened for eligibility by title and abstract. The full-text versions of the

remaining 15 eligible publications were evaluated in depth. Four publications were excluded from analysis (see Figure 1 for reasons), resulting in the identification of 11 eligible articles comprising 9 independent samples. Subsequently, authors of the selected publications were contacted to obtain original genotype and dimensional/categorical outcome data in addition to data available in the published manuscript. With the exception of two studies (both on samples with depressive disorders), original data could be obtained for all included publications upon author contact, thus allowing for *de novo* analyses. Additionally, three unpublished samples could be acquired, resulting in a total of 10 independent samples comprising 1,854 patients for baseline to post and 950 patients for additional FU data that were included in the main analysis (categorical analysis). For secondary dimensional analysis, data was available for 2,195 patients for pre to post comparison and 1,169 patients at FU. In six samples, panic disorder with/without agoraphobia constituted the main diagnosis. Two samples included social anxiety disorder as main diagnosis, and in two samples mixed anxiety disorder diagnoses were considered. Detailed study characteristics of all included samples are given in Table 2.

2.4 Study quality and risk of bias assessment

In order to assess the methodological quality and risk of bias in the included publications, a coding system based on a previous systematic review investigating neurobiological markers of treatment response (Lueken et al., 2016) was adopted addressing relevant study criteria that did not lead to study exclusion *per se* but may have an impact on the methodological study quality nonetheless. Methodological characteristics were quantified and a summative score was calculated (see Table 1 for scoring criteria). Sample size was coded as small, medium or large based on the sample size distribution by using tertiles. If available, information from primary clinical outcome articles were used supplementing information on study methodology.

2.5 Statistical analysis

Statistical analyses were computed with R v3.3 (R-Development-Core-Team 2009) and the package metafor v0.5-7 (Viechtbauer, 2010). Hardy-Weinberg equilibrium (HWE) of genotype distributions was approximated for all samples using Fisher's Exact test ($p \geq 0.05$). To account for ethnic discrepancies, calculations were performed first in each sample separately using Fisher's exact tests. For genotype comparisons, *5-HTT*LPR genotypes and those from the triallelic model *5-HTT*LPR/rs25531 were combined into a high-expression (L) group containing L_AL_A carriers versus a low-expression (S) group containing SS, S_AS_A, S_AS_G, S_GS_G, SL_G, SL_A, L_AL_G, and L_GL_G carriers (cf. Baffa et al., 2010; Baune et al., 2008; Schiele et al., 2020b; Schiele et al., 2016; Wendland et al., 2006).

Meta-Analysis

For joint analysis, all 10 samples were subjected to meta-analysis (N=1,854 for categorical and N=2,195 for dimensional analyses). Post-hoc sensitivity analyses indicate that the achieved sample size had a power to detect a genotype effect with the magnitude of d=0.2 with a power of 99%.

Treatment response analysis

For categorical analysis, treatment response was defined as a reduction of at least 50% in one of the respective primary outcome measures from baseline to post-treatment. For meta-analysis of the categorical baseline to post and follow-up outcome variables (responders vs non-responders), odds ratios (ORs) were determined as a measure for effect size. Q-statistic (Fleiss, 1981; Lau et al., 1997) was applied to assess heterogeneity. When effect sizes showed no heterogeneity, fixed-effects models (Mantel and Haenszel, 1959) were applied. In case of significant heterogeneity (I^2=Q-df/Q<0.05), random-effects models (DerSimonian and Laird, 1986) were calculated separately for *5-HTT*LPR and the triallelic design.

Dimensional Analysis

For dimensional analysis, mean differences in primary outcome measurement scores from pre- to post-treatment were considered. Meta-analysis on dimensional outcomes was performed as recommended in the R metafor package analysis example as described in (Morris, 2008) (http://www.metafor-project.org/doku.php/analyses:morris2008). For comparison of quantitative measures, the (bias-corrected) standardized mean change (Hedges'g) and sampling variance (v) within each genotype group (L and S) was computed with pretest, posttest and follow-up test means and standard deviations, using the metafor escalc() function as implemented in R. Calculation of the difference in the standardized mean change between the low (S) and high (L) expression groups ($g_{diff}=g_{low}-g_{high}$; $v_{diff}=v_{low}+v_{high}$) indicates how much larger the change in the low expression group was when compared to the high expression group. For meta-analysis, g_{diff} and v_{diff} values of all studies were passed to the rma () function computing random- and fixed-effects models.

3. RESULTS

3.1 Treatment response analysis

*5-HTT*LPR as well as the triallelic *5-HTT*LPR/rs25531 genotype frequencies for the high-expression (L) and low-expression (S) group are given in Table 3 for the whole sample and additionally stratified for medication status (with/without) per study, post-CBT assessment and after 6 or 12 months FU.

In accordance with the 5 published studies, no significant differences were observed when genotype frequencies of *5-HTT*LPR or the triallelic *5-HTT*LPR/rs25531 were compared between CBT-responder and non-responder in all three unpublished samples (Domschke et al., N=52, P_{best}=0.326; Schruers et al., N=96, P_{best}=0.456; Richter et al., N=78, P_{best}=0.458) post and 6 or 12 months after CBT.

When all 5 published and 3 unpublished samples were subjected to a fixed-effects based meta-analysis, neither the grouped L- nor the S- genotype was associated with treatment outcome immediately after (N=1,854; P_{LPR}=0.956, OR_{LPR}=0.99 [95% CI:0.81-0.121]; $P_{LPR/SNP}$=0.606, $OR_{LPR/SNP}$=1.08 [95% CI:0.83-1.41]) or 6 or 12 months after (N=950; P_{LPR}=0.876, OR_{LPR}=0.97 [95% CI:0.72-1.30]; $P_{LPR/SNP}$=0.704, $OR_{LPR/SNP}$=0.90 [95% CI:0.60-1.36]) CBT, respectively. The same was found when samples were analyzed separately depending on medication. Overall results did not change using a random-effects model. Results are listed in Table 3; for forest plots see Figure 2. Visual inspection of Funnel plots (Supplementary Figures S1 and S2) did not indicate the presence of publication bias.

3.2 Dimensional analysis

Means and standard deviation (SD) of psychometric scores at pre- and post- treatment as well as at FU as a function of the *5-HTT*LPR and the triallelic *5-HTT*LPR/rs25531 high-expression (L) and low-expression (S) group are given in Supplementary Tables S2 and S3 including subsamples, and stratified for medication (with/without). In line with the categorical assessment of *5-HTT*LPR on therapy response, the comparison of standardized pre-post and pre-FU mean changes (g) between the low (S) and the high (L) expression group showed rather small differences between both groups in the unpublished Schruers et al. (n_{post}=99, $g_{diff:highest}$=-0.286) and Richter et al. samples (n_{post}=81, $g_{diff:highest}$=0.209; n_{FU}=72, $g_{diff:highest}$=-0.114) post and 6 or 12 months after CBT-treatment. In contrast, differences of the standardized pre-post CBT-treatment mean changes in the unpublished sample by Domschke et al. (n_{post}=56) ranged from medium ($g_{diff:lowest}$=-0.383) to large effect size differences ($g_{diff:highest}$=-1.267) in the whole sample and the subsample without medication always with an 1.6 to 6.2-fold higher effect size for the high (L) and in patients with medication twice as high effect sizes for the low (S) expression group, For more detailed information see Supplementary Tables S2 and S3.

Meta-analysis of the 7 published and 3 unpublished studies in a fixed-effects model on the standardized mean changes of dimensional outcomes did not reveal any significant

differences between low (S) and high (L) expression groups - in concordance with the categorical CBT response analysis - neither at post-treatment (N=2,195; P_{LPR}=0.35, OR_{LPR}=0.93 [95% CI:0.80-1.01]; $P_{LPR/SNP}$=0.89, $OR_{LPR/SNP}$=1.01 [95% CI:0.83-1.25]) and the 6-month FU (N=1,169; P_{LPR}=0.74, OR_{LPR}=1.03 [95% CI:0.85-1.25]; $P_{LPR/SNP}$=0.13, $OR_{LPR/SNP}$=1.28 [95% CI:0.93-1.74]) for all samples not in the subsamples with medication (N=112; Post: P_{LPR}=0.59, OR_{LPR}=1.18 [95% CI:0.64-2.17]; $P_{LPR/SNP}$=0.59, $OR_{LPR/SNP}$=1.20 [95% CI:0.62-2.33]) and without medication (N=1,736; Post: P_{LPR}=0.54, OR_{LPR}=0.95 [95% CI:0.80-1.13]; $P_{LPR/SNP}$=0.96, $OR_{LPR/SNP}$=1.01 [95% CI:0.81-1.25]; N=954; FU: P_{LPR}=0.65, OR_{LPR}=1.05 [95% CI:0.84-1.32]; $P_{LPR/SNP}$=0.40, $OR_{LPR/SNP}$=1.14 [95% CI:0.84-1-55]). Results changed only slightly when a random-effects model was assumed. Detailed results are listed in Supplementary Tables S2 and S3; for forest plots see Supplementary Figures S3 and S4. Visual inspection of funnel plots (Supplementary Figures S5 and S6) did not argue for the presence of publication bias.

3.3 Study quality and risk of bias assessment

For all published samples, psychiatric exclusion criteria were reported in the respective publications. Two (28.6%) additionally reported somatic exclusion criteria. Comorbid diagnoses were allowed in four (57.1%) of assessed samples and excluded in two (28.6%). One study did not report on comorbidities. Concomitant medication in addition to psychotherapeutic treatment was allowed in five (71.4%) samples; for two (28.6%), information on medication was not reported. Potential confounders were analyzed and, if applicable, statistically controlled for in all samples (100%). *5-HTT* rs25531 was analyzed in three (42.9%) samples. Three of the published samples (42.9%) reported a 6-month FU assessment. Finally, adherence to RCT-methodology as the gold standard in clinical research was evaluated. A primary outcome was defined and used in the respective analyses in all but one (85.7%) samples. The outcome measure was assessed using a clinician-rated instrument in three (42.9%) and a self-report instrument in four (57.1%) samples. In four samples (57.1%), treatment was randomized and a comparator-control was employed.

4. DISCUSSION

The present study constitutes a meta-analysis addressing the association of *5-HTT*LPR with CBT outcome in anxiety disorders. Comprising a total of 2,195 patients from ten independent samples (including three unpublished samples), no evidence was found that the *5-HTT*LPR genotype, either of *5-HTT*LPR alone or in combination with the functionally related single nucleotide polymorphism rs25531, can be discerned as a moderator on response to CBT outcome in anxiety disorders. This held true for comparisons at post-treatment time points and at follow-up. Secondary analyses including medication status revealed no differences with regard to intake of psychopharmacological medication.

The present findings add to the recurring debate within the larger framework of gene-environment (GxE) research on the role of *5-HTT*LPR in the conferral of disorder risk by influencing sensitivity to environmental circumstances. *5-HTT*LPR has been a central focus in GxE research following a landmark study by Caspi et al. (2003) investigating its interaction with childhood maltreatment on depression. Since then, a variety of environmental factors – both positive and negative – have been addressed as to whether they can increase or decrease susceptibility to disease depending on genotype. However, results have been mixed, with several meta-analyses arguing either for or against the interaction (Karg et al., 2011; Munafo et al., 2009; Risch et al., 2009; Sharpley et al., 2014). Addressing methodological concerns of previous analyses, the most recent collaborative meta-analysis (Culverhouse et al., 2018) on the interaction of *5-HTT*LPR and stress in the conferral of depression in a total of 43,165 subjects has found no evidence for *5-HTT*LPR to interact with environmental influences, concluding that there is likely no true interaction effect or if so, it is a very small effect, only applicable to specific circumstances and not broadly generalizable. The present results – conceptualizing the GxE model in the context of CBT constituting a positive environmental influence – argue in the same direction by providing additional negative evidence for *5-HTT*LPR to moderate sensitivity to non-genetic external influences.

However, as treatment studies necessary to detect therapygenetic effects usually are smaller-scale, the statistical power of the present meta-analysis may be insufficient to detect such small effects and should be updated by larger studies in the future.

While the results by Culverhouse et al. (2018) did not yield an association with *5-HTT*LPR genotype, they reported a significant influence of stress on depression risk independent of genotype. In a similar vein, occurrence of significant life events has also been linked to an increased risk for anxiety disorders and has been shown to often precede disorder onset (Fernandes and Osorio, 2015; Klauke et al., 2010), pointing to the clinical relevance of efforts aiming at reducing stressors themselves or to counteract the long-term negative effects conferred by environmental insults, for instance by strengthening protective factors (cf. (Schiele et al., 2020c)) in the prevention of anxiety disorders or in the context of psychotherapy following disorder onset in clinical populations.

It has to be noted, however, that FU data as well as medication and rs25531 genotype information was available only for subsamples, thus further limiting the statistical power and representativeness as compared to the main analysis (pre-post). Also, the positive effect reported by Eley et al. (2012) emerged at the 6-month FU mark only, but not immediately following treatment. Therefore, it cannot be excluded that in contrast to short-term effects at the post-treatment mark, initial changes conveyed by CBT may unfold genotype-dependent effects in the interaction between new coping strategies and the respective environment later on. Thus, future studies should particularly focus on addressing long-term changes following initial CBT.

Epigenetic mechanisms such as DNA methylation have been shown to crucially modify gene function and to be related to both anxiety disorder susceptibility and treatment response (for review see Schiele and Domschke, 2018; Schiele et al., 2020a). In particular, differential *5-HTT* promoter methylation has been demonstrated to predict response to pharmacotherapy (Domschke et al., 2014) or to be related to successful CBT response (Roberts et al., 2014). Future studies are needed to address whether the discrepant findings reported in the

literature on putative therapygenetic effects of the *5-HTT*LPR are moderated by epigenetic changes such as DNA methylation status of the respective gene promoter region.

Since anxiety disorders are polygenic disorders, comprising the interplay of several different genes of small individual effect, haplotypic or epistatic effects should be taken into account in the search for predictive biomarkers of therapy response. For instance, gene-gene interactions between serotonin pathway genes or of serotonergic genes with other transmitter systems have been shown to modulate panic disorder risk, and, in a similar vein, to further influence GxE interactions interactions (cf. Freitag et al., 2006; Grabe et al., 2012; Strug et al., 2010). However, in recent years, the focus of psychiatric genetic research has shifted to hypothesis-free, genome-wide association studies (GWAS) over classical candidate gene studies, citing inadequate power due to small sample sizes, high rates of false positive findings and publication bias as the leading cause for the lack of replication of the proposed candidate genes in genome-wide approaches in psychiatry (cf. Border et al., 2019; Border and Keller, 2017; Koenen et al., 2013). Small scale GWAS analyses in relation to anxiety disorders and treatment response have resulted in only limited suggestive finding thus far, which, however, indeed did not provide evidence for commonly studied candidate gene polymorphisms such as *5-HTT*LPR to be associated with behavioral outcomes above chance level. Here, post-hoc sensitivity analyses indicate that the achieved sample size allowed for the detection of a small effect (d=0.2) with adequate statistical power (99%), indicating that if a true effect of *5-HTT*LPR on treatment outcome existed, it would only be of very small magnitude However, given the polygenic nature of anxiety disorders comprising the cumulative effect of many genes of only small individual impact (d<.02), employing whole genome and polygenic risk score (PRS) approaches in larger, homogenous samples are warranted as a highly promising future direction in therapygenetic research.

With regard to ancestry, all participants included in the present study were almost exclusively of Caucasian background, which in itself can be considered advantageous as it decreased genetic heterogeneity, however, it limits generalizability to non-Caucasian populations.

No sub-group analyses stratified by specific anxiety disorders were possible since the majority of samples included in the present analyses comprised patients with panic disorder with or without agoraphobia, while two samples included patients with social anxiety disorder and two samples with mixed anxiety diagnoses. Therefore, generalization to other classes of anxiety disorders should be done cautiously.

In conclusion, the present results do not support the hypothesis of *5-HTT*LPR as a moderator of treatment outcome for CBT in anxiety disorders. Future studies including GWAS (cf. Coleman et al., 2016) and PRS approaches that better capture the multivariate nature of multiple vulnerability genes are needed to investigate therapygenetic effects. Future studies may help to clarify whether other factors such a long-term behavioral changes or epigenetic factors may explain further variance in these complex gene-environment interactions and molecular-genetic pathways that may confer behavioral change following psychotherapy.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support during literature search by Stefanie L. Kunas.

CONFLICT OF INTEREST

AR reports speaker honoraria and advisory board honoraria from Janssen, Servier, Medice, Shire and neuraxpharm. VA has received compensations for his contributions as member of advisory boards and for presentations for the following companies: Astra-Zeneca, Eli Lilly, Janssen-Cilag, Lundbeck, Otsuka, Sanofi, Servier, and Trommsdorff. TCE is part funded by a program grant from the UK Medical Research Council (MR/M021475/1), and 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. PP reports having received research funding from Volkswagenstiftung (A124277) and that he is shareholder of a commercial company (VTplus) that develops virtual reality research

systems. KD is a member of the Janssen Pharmaceuticals, Inc. Steering Committee Neurosciences. All other authors reported no biomedical financial interests or potential conflicts of interest.

ROLE OF FUNDING SOURCE

This work was supported by the German Research Foundation (DFG) – project number 44541416 – TRR 58, projects C02 (to KD), C09 (to UL) and Z02 (to AR, KD, PP, TBL and UL), SFB 1193 Z03 (to AR), and the German Ministry of Research and Education (BMBF, 01EE1402F, PROTECT-AD, P5 to KD).

This work was partly funded by a grant from the UK Medical Research Council to TC Eley (G0901874).

The study is in part funded by the German Federal Ministry of Education and Research (BMBF, 01GV0614) as part of the larger BMBF Psychotherapy Research Funding Initiative Improving the Treatment of Panic Disorder.

None of the funding agencies had a further role in study design, in the collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the paper for publication.

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FOOTNOTES

[a] The initial search also included primary diagnosis of depression (search terms are given in Table S1), yielding two additional articles on the effect of *5-HTT*LPR on CBT outcome in major depressive disorder. However, since original data could not be obtained for either article, they were excluded from the present analysis, resulting in the consideration of anxiety disorders only.

TABLES

Table 1. Risk of bias assessment coding system.

Marker keywords	1 point	0.5 points	0 points
Sample			
Sample size	L	M	S
Confounder control			
Exclusion criteria (psychiatric)	Reported	-	Not reported
Exclusion criteria (somatic)	Reported	-	Not reported
Comorbidity assessment	Reported – yes	Reported – no	Not reported
Concomitant medication	Reported – yes	Reported – no	Not reported
Inclusion of rs25531	Yes	-	Not reported
Confounder analysis	Reported – yes	Reported – no	Not reported
Statistical control	Yes or no confounders	-	Not reported
Outcome measure			
Primary outcome defined	Yes	-	No
Applied in present analysis	Yes	-	No
Clinical or self-rated	Clinician	Self	-
Evidence-based treatment (Bandelow et al. 2014)	Yes	-	No
Study design			
Comparator	Active	Waitlist	No
Randomization	Yes	-	No

Legend to Table 1. Sample sizes are coded based on the sample size distribution by using tertiles, L: large (N > 318); M: medium (112 < N ≤ 318); S: small (N ≤ 112).

Table 2. Sample characteristics

#	Author	Year	AD	Description	Duration	Age group	Ethnicity	Trial size	N analysis	Confounder control		Outcome definition		Clinical response criterion			*5-HTT*LPR +/- rs25531	Timepoint outcome assessment
										Psychiatric exclusion criteria	Concomitant medication	Primary study outcome	Current outcome measure	categorical	dimensional	Comparator		
1	Knuts et al. (34)	2014	PD + AG	CBT	1 week	adult	Caucasian	99	99	severe depressive disorder, suicidal intent, psychosis, substance abuse, cognitive impairment	Antidepressants	FQ-AGO (pre-post)	FQ-AGO, PAS, MADRS	FQ-AGO 50% reduction (pre-post)	FQ-AGO mean difference (pre-post)	no	*5-HTT*LPR +rs25531	post
2	Anders-son et al. (30)	2013	SAD	i-CBT vs. g-CBT	study 1: 15 weeks, study 2: 9 weeks	adult	Caucasian	330 (2 studies)	314	current substance abuse, history of psychosis or bipolar disorder, severe depression, suicidal ideation (study 1: cluster A or B PED also excluded)	SSRIs, SNRIs (study 1); antidepressants (study 2)	LSAS (reliable change index)	LSAS	-	LSAS mean difference (pre-post, FU)	i-CBT vs. g-CBT	*5-HTT*LPR -rs25531	post; study 1: 6 month FU: study 2: 1 year FU
3	Lonsdorf et al. (31)	2010	PD +/- AG	i-CBT vs. g-CBT	10 weeks	adult	Caucasian	87	69	severe depression or suicidal ideation	Antidepressants; benzodiazepines	not defined	HADS	HADS 50% reduction (pre-post)	HADS mean difference (pre-post)	i-CBT vs. g-CBT	*5-HTT*LPR +rs25531	cognitive block wks 1-3, exposure block wks 4-9

4	Lueken et al. (33)	2015	PD + AG	T+ CBT vs. T-CBT	6 weeks	adult	Cauca-sian	369	231	suicidal intent, psychotic or bipolar disorder, borderline personality disorder, current alcohol dependence	No	HAM-A	HAM-A	HAM-A 50% reduction (pre-post)	HAM-A mean difference (pre-post, FU)	T+ CBT vs. T-CBT	*5-HTT*LPR +rs25531	Post
5	Eley et al. (9)	2012	any AD	CBT	4-12 sess. (dep. on study)	children	Cauca-sian	584 (6 studies)	359	intellectual impairment, psychosis	not reported	absence of primary AD (ADIS-IV-C/P)	absence of primary and any AD	absence of primary AD (ADIS Score < 4)	ADIS mean difference (pre-post, FU)	no	*5-HTT*LPR -rs25531	post, 3, 6, or 12 months FU
6	Lester et al. (32)	2016	any AD	CBT	8-25 sess. (dep. on study)	children	67,5% Caucasian	829	829	physical/ intellectual impairment, psychoses, concurrent treatment	not reported	absence of primary AD	absence of primary and any AD	absence of primary AD (ADIS Score < 4)	ADIS mean difference (pre-post, FU)	no	5-HTTLPR +SNP	post; 3, 6, or 12 months FU
7	Domschke et al. unpublished		PD + AG	CBT	6 weeks	adult	Caucasian		56	see Ziegler et al. 2016	see Ziegler et al. 2016	see Ziegler et al. 2016	HAM-A	HAMA-A 50% reduction (pre-post)	HAM-A mean difference (pre-post)	no	*5-HTT*LPR +rs25531	post
8	Schruers et al. unpublished		PD + AG	CBT		adult	Caucasian		99		Antidepressants	FQ-AGO	FQ-AGO	FQ-AGO 50% reduction (pre-post)	FQ-AGO mean difference (pre-post)	no	*5-HTT*LPR +rs25531	post
9	Richter et al. unpublished		PD + AG	CBT		adult	Caucasian	124	92		No	HAM-A, CGI, MI, PAS	HAM-A	HAM-A 50% reduction (pre-post)	HAM-A mean difference (pre-post, FU)		*5-HTT*LPR +rs25531	Post, 6 months FU

Legend to Table 2. AD: anxiety disorder; PD: panic disorder; AG: agoraphobia; SAD: social anxiety disorder; CBT: cognitive behavioral therapy; i-CBT: individual cognitive behavioral therapy; g-CBT: group cognitive behavioral therapy; T+ CBT: CBT with therapist-guided exposure sessions; T- CBT: CBT with non-guided exposure sessions; SSRI: selective serotonin reuptake inhibitor; SNRI: serotonin-norepinephrine reuptake inhibitor; FQ-AGO: Fear Questionnaire, agoraphobia score; PAS: Panic and Agoraphobia Scale; MADRS: Montgomery-Asberg Depression Rating Scale; LSAS: Liebowitz Social Anxiety Scale; HAM-A: Hamilton Anxiety Rating Scale; ADIS-IV-C/P: Anxiety Disorders Interview Schedule Child/Parent Version.

Table 3. Treatment response analysis

	Clinical effect post-treatment						Clinical effect 6 or 12 month follow-up					
	Total		with medication		without medication		Total		with medication		without medication	
	*5-HTT*LPR	*5-HTT*LPR/ rs25531	*5-HTT*LPR	*5-HTT*LPR/ rs25531	*5-HTT*LPR	*5-HTT*LPR/ rs25531	*5-HTT*LPR	*5-HTT*LPR/ rs25531	*5-HTT*LPR	*5-HTT*LPR/ rs25531	*5-HTT*LPR	*5-HTT*LPR/ rs25531
	LL vs. SS/SL	L_AL_A vs. SS, $SL_G/SL_A/$ L_AL_G/L_GL_G	LL vs. SS/SL	L_AL_A vs. SS, $SL_G/SL_A/$ L_AL_G/L_GL_G	LL vs. SS/SL	L_AL_A vs. SS, $SL_G/SL_A/$ L_AL_G/L_GL_G	LL vs. SS/SL	L_AL_A vs. SS, $SL_G/SL_A/$ L_AL_G/L_GL_G	LL vs. SS/SL	L_AL_A vs. SS, $SL_G/SL_A/$ L_AL_G/L_GL_G	LL vs. SS/SL	L_AL_A vs. SS, $SL_G/SL_A/$ L_AL_G/L_GL_G
Lonsdorf et al., 2010 (Non-responders: N=23; Responders: N=32)							**(Non-responders: N=0; Responders: N=0)**					
Non-responders	5/18	2/21	2/9	0/11	3/9	2/10	--	--	--	--	--	--
Responders	9/23	5/27	4/7	2/9	5/16	3/18	--	--	--	--	--	--
P-value	0.756	0.686	0.635	0.476	1.000	1.000	--	--	--	--	--	--
Eley et al., 2012 (Non-responders: N=203; Responders: N=209)							**(Non-responders: N=128; Responders: N=219)**					
Non-responders	68/135	--	--	--	68/135	--	37/91	--	--	--	37/91	--
Responders	65/144	--	--	--	65/144	--	75/144	--	--	--	75/144	--
P-value	0.674	--	--	--	0.674	--	0.342	--	--	--	0.342	--
Andersson et al., 2013; study 1 (Non-responders: N=0; Responders: N=0)							**(Non-responders: N=0; Responders: N=0)**					
Non-responders	--	--	--	--	--	--	--	--	--	--	--	--
Responders	--	--	--	--	--	--	--	--	--	--	--	--
P-value	--	--	--	--	--	--	--	--	--	--	--	--
Andersson et al., 2013; study 2 (Non-responders: N=0; Responders: N=0)							**(Non-responders: N=0; Responders: N=0)**					
Non-responders	--	--	--	--	--	--	--	--	--	--	--	--
Responders	--	--	--	--	--	--	--	--	--	--	--	--
P-value	--	--	--	--	--	--	--	--	--	--	--	--
Knuts et al.,2014 (Non-responders: N=26; Responders: N=75)							**(Non-responders: N=0; Responders: N=0)**					
Non-responders	14/12	12/14	5/1	5/1	7/7	6/8	--	--	--	--	--	--
Responders	28/47	22/53	7/14	7/14	16/17	13/20	--	--	--	--	--	--
P-value	0.169	0.150	0.060	0.060	1.000	1.000	--	--	--	--	--	--
Lueken et al., 2015 (Non-responders: N=86; Responders: N=109)							**(Non-responders: N=50; Responders: N=127)**					
Non-responders	28/58	22/63	--	--	28/58	22/63	18/32	15/35	--	--	18/32	15/35
Responders	35/74	32/77	--	--	35/74	32/77	38/89	33/93	--	--	38/89	33/93
P-value	1.000	0.631	--	--	1.000	0.631	0.475	0.708	--	--	0.475	0.708
Lester et al., 2016 (Non-responders: N=345; Responders: N=520)							**(Non-responders: N=101; Responders: N=255)**					
Non-responders	89/256	56/238	--	--	89/256	56/238	32/69	23/69	--	--	32/69	23/69
Responders	144/376	96/356	--	--	144/376	96/356	65/190	48/179	--	--	65/190	48/179
P-value	0,584	0.515	--	--	0.584	0.515	0.238	0.461	--	--	0.238	0.461
Domschke et al. unpublished (Non-responders: N=29; Responders: N=23)							**(Non-responders: N=0; Responders: N=0)**					
Non-responders	9/20	9/20	4/10	4/10	5/9	5/9	--	--	--	--	--	--
Responders	7/16	5/18	2/12	1/13	5/4	4/5	--	--	--	--	--	--

P-value	1.000	0.539	0.648	0.326	0.417	1.000	--	--	--	--	--	--
Schruers et al. unpublished (Non-responders: N=34; Responders: N=62)							**(Non-responders: N=0; Responders: N=0)**					
Non-responders	13/21	9/25	5/8	3/10	8/13	6/15	--	--	--	--	--	--
Responders	25/37	22/40	8/12	8/12	17/25	14/28	--	--	--	--	--	--
P-value	1.000	0.494	1.000	0.456	1.000	0.780	--	--	--	--	--	--
Richter et al. unpublished (Non-responders: N=52; Responders: N=26)							**(Non-responders: N=43; Responders: N=27)**					
Non-responders	21/31	19/33	--	--	21/31	19/33	17/26	15/28	--	--	17/26	15/28
Responders	10/16	10/16	--	--	10/16	10/16	12/15	12/15	--	--	12/15	12/15
P-value	1.000	1.000	--	--	1.000	1.000	0.804	0.458	--	--	0.804	0.458
Total (Non-responders: N=798; Responders: N=1056)							**Total (Non-responders: N=322; Responders: N=628)**					
Non-responders	247/551	129/414	16/28	12/32	229/518	116/376	104/218	53/132	--	--	104/218	53/132
Responders	323/733	192/587	21/45	18/48	297/672	172/520	190/438	93/287	--	--	190/438	93/287
Heterogeneity:												
P-value	0.873	0,562	0.172	0.053	0.981	1.000	0.368	0.544	--	--	0.368	0.544
Cochran-Mantel-Haenszel Metaanalysis:												
Fixed effect	0,99	1,08	0,67	0.73	1,02	1,14	0.97	0.90	--	--	0.97	0.90
P-value	0.956	0,606	0.465	0.639	0.864	0.397	0.876	0.704	--	--	0.876	0.704
DerSimonian and Laird Metaanalysis:												
Random effect	0,99	1,08	0,67	0,68	1,02	1,14	0.96	0.90	--	--	0.96	0.90
P-value	0.912	0,576	0.512	0.674	0.825	0.359	0.794	0.622	--	--	0.794	0.622

Legend to Table 3. Association results for the *5-HTT*LPR as well as the triallelic *5-HTT*LPR/rs25531 genotype per study, followed by meta-analysis. Table shows for each model high-expression (L) and low-expression (S) group counts for non-responder and responder, as well as the corresponding *P* -values of the whole sample and subsamples, stratified for medication status (with/without), post-CBT assessment and after 6 or 12 months follow-up. Further, total non-responder and responder counts are given for the *5-HTT*LPR as well as the triallelic *5-HTT*LPR/rs25531 model, *P*-values for heterogeneity, odds ratios and *P*-values of the fixed and random effects meta-analysis.

FIGURES

Figure 1

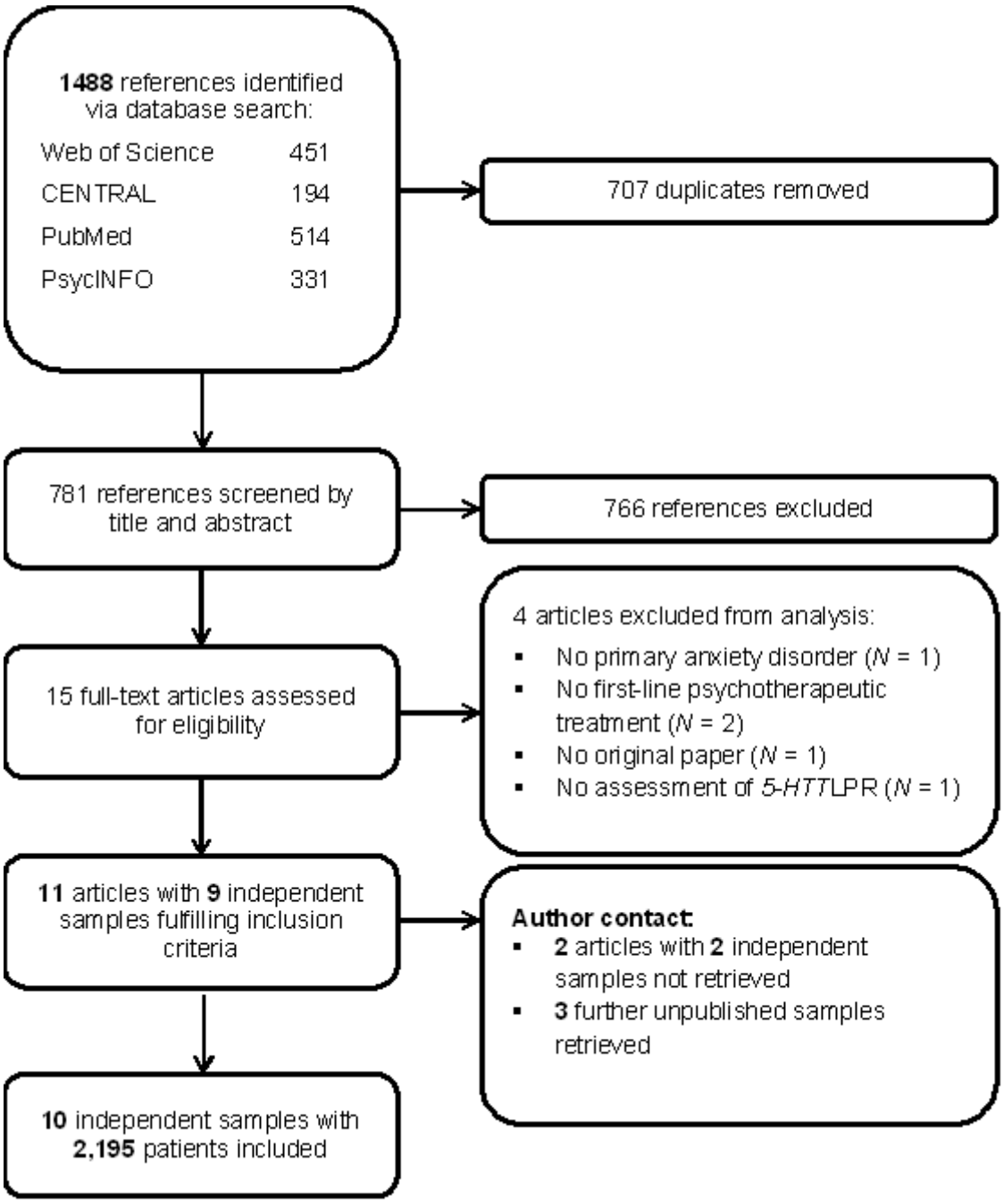

Figure 2

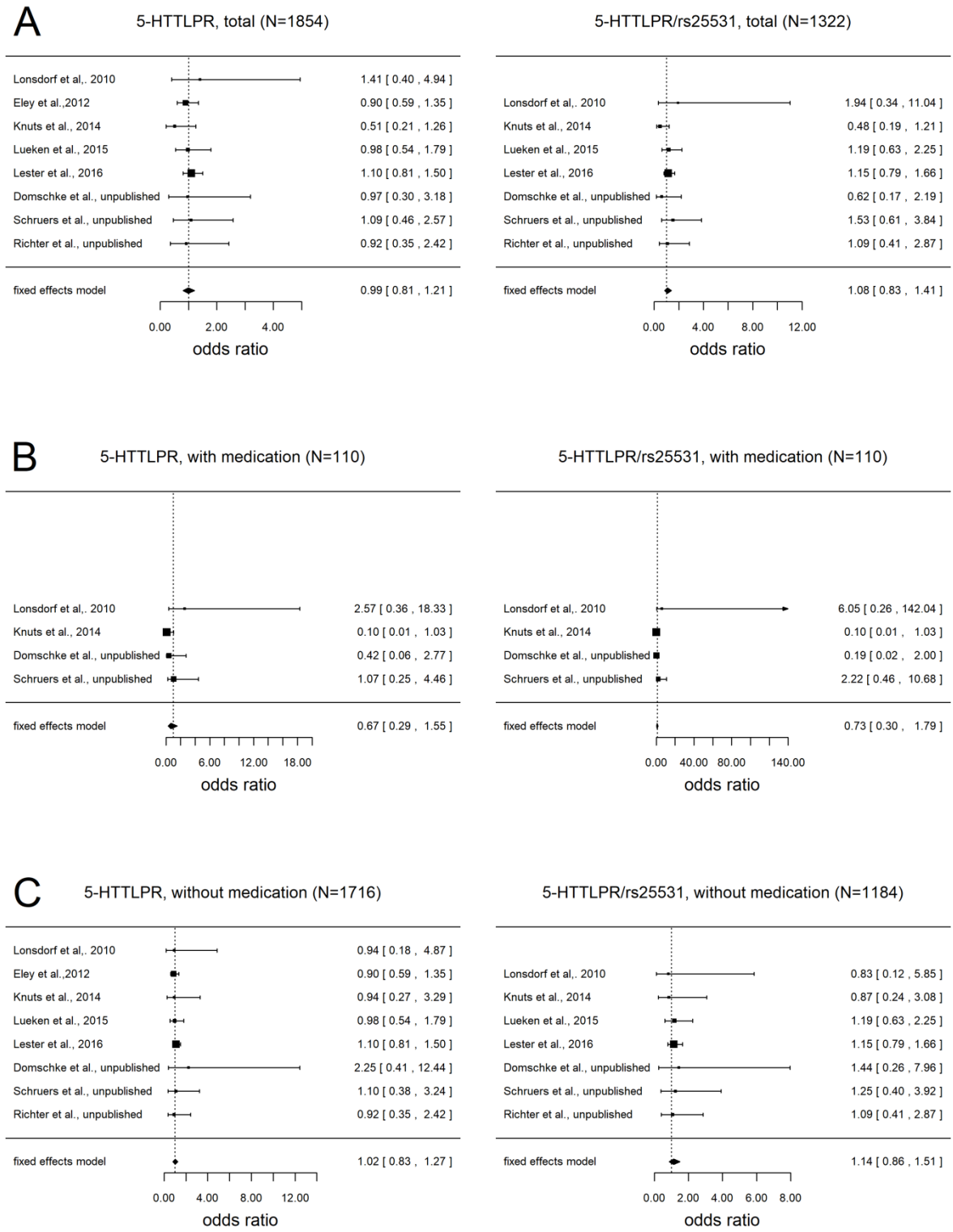
A
5-HTTLPR, total (N=1854)
Lonsdorf et al,. 2010 1.41 [0.40 , 4.94]
Eley et al.,2012 0.90 [0.59 , 1.35]
Knuts et al., 2014 0.51 [0.21 , 1.26]
Lueken et al., 2015 0.98 [0.54 , 1.79]
Lester et al., 2016 1.10 [0.81 , 1.50]
Domschke et al., unpublished 0.97 [0.30 , 3.18]
Schruers et al., unpublished 1.09 [0.46 , 2.57]
Richter et al., unpublished 0.92 [0.35 , 2.42]
fixed effects model 0.99 [0.81 , 1.21]
0.00 2.00 4.00
odds ratio
5-HTTLPR/rs25531, total (N=1322)
Lonsdorf et al,. 2010 1.94 [0.34 , 11.04]
Knuts et al., 2014 0.48 [0.19 , 1.21]
Lueken et al., 2015 1.19 [0.63 , 2.25]
Lester et al., 2016 1.15 [0.79 , 1.66]
Domschke et al., unpublished 0.62 [0.17 , 2.19]
Schruers et al., unpublished 1.53 [0.61 , 3.84]
Richter et al., unpublished 1.09 [0.41 , 2.87]
fixed effects model 1.08 [0.83 , 1.41]
0.00 4.00 8.00 12.00
odds ratio
B
5-HTTLPR, with medication (N=110)
Lonsdorf et al,. 2010 2.57 [0.36 , 18.33]
Knuts et al., 2014 0.10 [0.01 , 1.03]
Domschke et al., unpublished 0.42 [0.06 , 2.77]
Schruers et al., unpublished 1.07 [0.25 , 4.46]
fixed effects model 0.67 [0.29 , 1.55]
0.00 6.00 12.00 18.00
odds ratio
5-HTTLPR/rs25531, with medication (N=110)
Lonsdorf et al,. 2010 6.05 [0.26 , 142.04]
Knuts et al., 2014 0.10 [0.01 , 1.03]
Domschke et al., unpublished 0.19 [0.02 , 2.00]
Schruers et al., unpublished 2.22 [0.46 , 10.68]
fixed effects model 0.73 [0.30 , 1.79]
0.00 40.00 80.00 140.00
odds ratio
C
5-HTTLPR, without medication (N=1716)
Lonsdorf et al,. 2010 0.94 [0.18 , 4.87]
Eley et al.,2012 0.90 [0.59 , 1.35]
Knuts et al., 2014 0.94 [0.27 , 3.29]
Lueken et al., 2015 0.98 [0.54 , 1.79]
Lester et al., 2016 1.10 [0.81 , 1.50]
Domschke et al., unpublished 2.25 [0.41 , 12.44]
Schruers et al., unpublished 1.10 [0.38 , 3.24]
Richter et al., unpublished 0.92 [0.35 , 2.42]
fixed effects model 1.02 [0.83 , 1.27]
0.00 4.00 8.00 12.00
odds ratio
5-HTTLPR/rs25531, without medication (N=1184)
Lonsdorf et al,. 2010 0.83 [0.12 , 5.85]
Knuts et al., 2014 0.87 [0.24 , 3.08]
Lueken et al., 2015 1.19 [0.63 , 2.25]
Lester et al., 2016 1.15 [0.79 , 1.66]
Domschke et al., unpublished 1.44 [0.26 , 7.96]
Schruers et al., unpublished 1.25 [0.40 , 3.92]
Richter et al., unpublished 1.09 [0.41 , 2.87]
fixed effects model 1.14 [0.86 , 1.51]
0.00 2.00 4.00 6.00 8.00
odds ratio

Figure 3

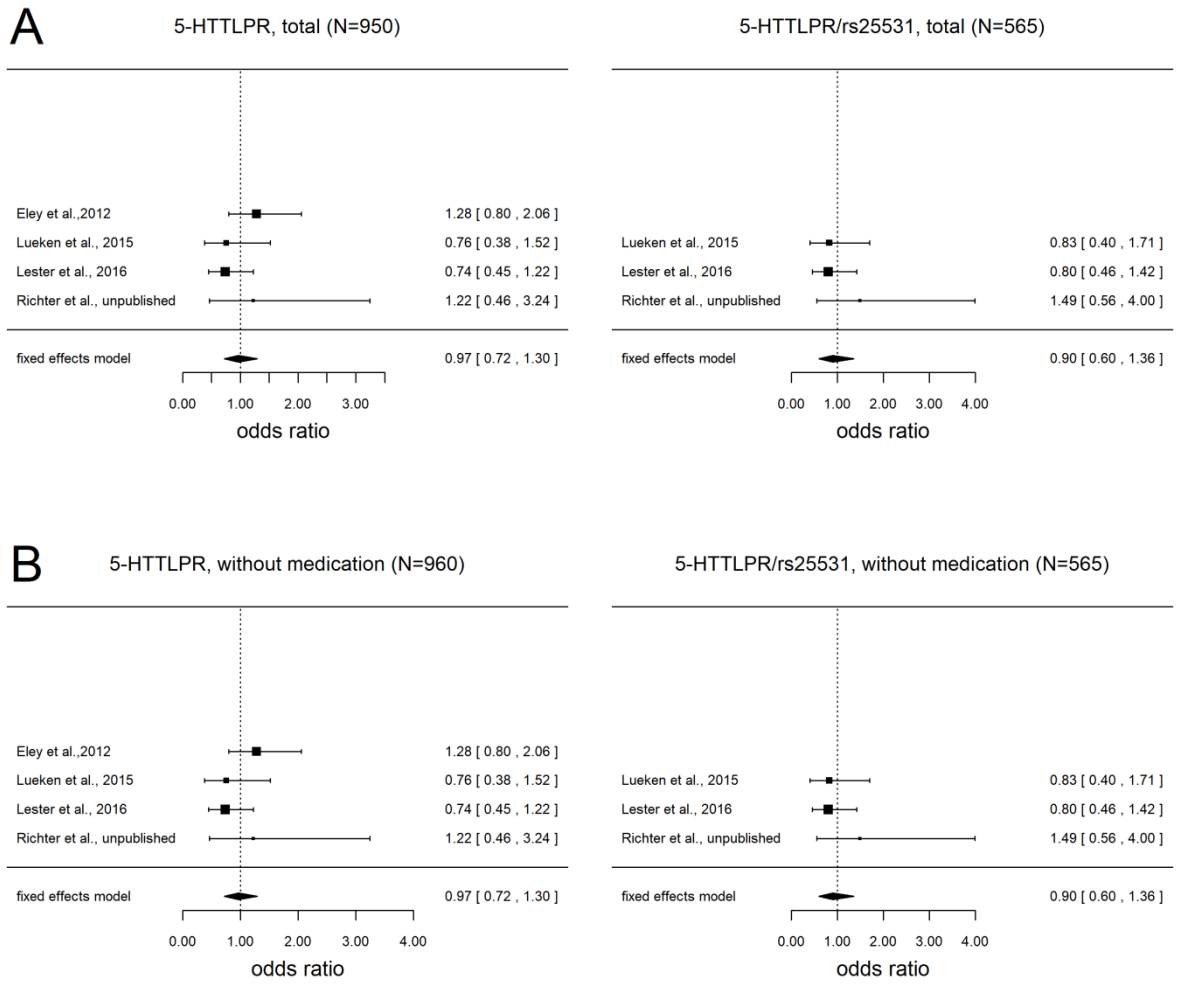
A
5-HTTLPR, total (N=950)
Eley et al.,2012
Lueken et al., 2015
Lester et al., 2016
Richter et al., unpublished
fixed effects model
1.28 [0.80 , 2.06]
0.76 [0.38 , 1.52]
0.74 [0.45 , 1.22]
1.22 [0.46 , 3.24]
0.97 [0.72 , 1.30]
0.00 1.00 2.00 3.00
odds ratio
5-HTTLPR/rs25531, total (N=565)
Lueken et al., 2015
Lester et al., 2016
Richter et al., unpublished
fixed effects model
0.83 [0.40 , 1.71]
0.80 [0.46 , 1.42]
1.49 [0.56 , 4.00]
0.90 [0.60 , 1.36]
0.00 1.00 2.00 3.00 4.00
odds ratio
B
5-HTTLPR, without medication (N=960)
Eley et al.,2012
Lueken et al., 2015
Lester et al., 2016
Richter et al., unpublished
fixed effects model
1.28 [0.80 , 2.06]
0.76 [0.38 , 1.52]
0.74 [0.45 , 1.22]
1.22 [0.46 , 3.24]
0.97 [0.72 , 1.30]
0.00 1.00 2.00 3.00 4.00
odds ratio
5-HTTLPR/rs25531, without medication (N=565)
Lueken et al., 2015
Lester et al., 2016
Richter et al., unpublished
fixed effects model
0.83 [0.40 , 1.71]
0.80 [0.46 , 1.42]
1.49 [0.56 , 4.00]
0.90 [0.60 , 1.36]
0.00 1.00 2.00 3.00 4.00
odds ratio

FIGURE LEGENDS

Legend to Figure 1: Flow chart for study inclusion

Legend to Figure 2: Forest plot on therapygenetic effects of the 5-HTTLPR polymorphism and clinical response rates at post-treatment (Cochran-Mantel-Haenszel meta-analysis). Forest plots of *5-HTT*LPR as well as the triallelic *5-HTT*LPR/rs25531 in the total sample (A), as well as the subsamples with (B) and without (C) medication at post treatment.

Legend to Figure 3: Forest plot on therapygenetic effects of the 5-HTTLPR polymorphism and clinical response rates at follow-up (Cochran-Mantel-Haenszel meta-analysis). Forest plots of *5-HTT*LPR as well as the triallelic *5-HTT*LPR/rs25531 in the total sample (A), as well as the subsamples without (B) medication at 6 or 12 month follow-up.