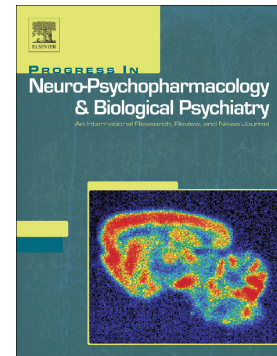# Accepted Manuscript

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Imaging genetics Paradigms in Depression Research: Systematic Review and Meta-analysis

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Supplementary online material

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Abstract

Imaging genetics studies involving participants with major depressive disorder (MDD) have expanded. Nevertheless, findings have been inconsistent. Thus, we conducted a systematic review and meta-analysis of imaging genetics studies that enrolled MDD participants across major databases through June 30th, 2017. Sixty-five studies met eligibility criteria (N=4034 MDD participants and 3293 controls), and there was substantial between-study variability in the methodological quality of included studies. However, few replicated findings emerged from this literature with only 22 studies providing data for meta-analyses (882 participants with MDD and 616 controls). Total hippocampal volumes did not significantly vary in MDD participants or controls carrying either the *BDNF Val66Met* 'Met' (386 participants with MDD and 376 controls) or the *5-HTTLPR* short 'S' (310 participants with MDD and 230 controls) risk alleles compared to non-carriers. Heterogeneity across studies was explored through meta-regression and subgroup analyses. Gender distribution, the use of medications, segmentation methods used to measure the hippocampus, and age emerged as potential sources of heterogeneity across studies that assessed the association of *5-HTTLPR* short 'S' alleles and hippocampal volumes. Our data also suggest that the methodological quality of included studies, publication year, and the inclusion of brain volume as a covariate contributed to the heterogeneity of studies that assessed the association of the *BDNF Val66Met* 'Met' risk allele and hippocampal volumes. In exploratory voxel-wise meta-analyses, MDD participants carrying the *5-HTTLPR* short 'S' allele had white matter microstructural abnormalities predominantly in the corpus callosum, while carriers of the *BDNF Val66Met* 'Met' allele had larger grey matter volumes and hyperactivation of the right middle frontal gyrus compared to non-carriers. In conclusion, few replicated findings emerged from imaging genetics studies that included participants with MDD. Nevertheless, we explored and identified specific sources of

heterogeneity across studies, which could provide insights to enhance the reproducibility of this emerging field.

Keywords: depression; genetic polymorphisms; diffusion tensor imaging; magnetic resonance imaging; voxel-based morphometry; meta-analysis

Abbreviations

AD, axial diffusivity; MDD, Major depression disorder; MRI, magnetic resonance imaging; MD, mean diffusivity; SDM, Seed-based *d* Mapping; VBM, voxel-based morphometry; fMRI, functional MRI; HC, healthy controls; ES, effect size; DTI, diffusion-tensor imaging; FA, fractional anisotropy; FWHM, full width at half maximum; OFC, orbitofrontal cortex; UF, uncinated fasciculus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; BDNF, brain-derived neurotrophic factor; COMT, catechol-o-methyl transferase; 5-HTTLPR, *5-HTT* gene (*SLC6A4*) linked polymorphic region; DLPFC, dorsolateral prefrontal cortex; VLPFC, ventrolateral prefrontal cortex; SLF, superior longitudinal fasciculus; ILF, inferior longitudinal fasciculus; ROI, region of interest; HC, healthy controls; WBA, whole brain analysis; RCs, carriers of risk genotypes; NRCs, non-carriers of risk genotypes; DMN, default mode network; TBSS, tract-based spatial statistics.

1. Introduction

Major depressive disorder (MDD) is a chronic condition with an estimated lifetime prevalence of 14.6% in high-income countries and 11.1% in low and middle-income countries. The disorder is a leading source of disability worldwide (Bromet et al., 2011; Zhang et al., 2016). The pathophysiology of depression remains incompletely understood and may involve the complex interaction of genetic and environmental factors (Otte et al., 2016; Vialou et al., 2013). The heritability of depression has been estimated to be 37% according to twin studies (Sullivan et al., 2000). However, the search for genetic risk variants for depression has been challenging, partially due to the phenotypic heterogeneity of this syndrome that may imply distinct genetic architectures (Kendler, 2016; Mullins and Lewis, 2017). Only with the recent development of genome-wide approaches and the inclusion of large samples sizes, the field has witnessed the identification of the first genetic variants of risk for depression that reached genome-wide significance (CONVERGE consortium, 2015; Direk et al., 2017; Hyde et al., 2016; Mullins and Lewis, 2017).

Imaging genetics is a field of investigation that aims to gain mechanistic insights on the impact of genetic variations on brain structure, chemistry, and function in health and disease (Carter et al., 2017; Pereira et al., 2017). Several approaches can be used in imaging genetics studies. In the *candidate gene approach*, a hypothesis-driven association with a genetic variation is related to neuroimaging measures. This approach also includes genetic variants derived from a genome-wide association study (GWAS), where the identified variants can be further investigated for possible influences in brain structure or function, theoretically enhancing the degree of specificity and confidence of the hypothesis. A second approach is the *discovery-based approach* (Carter et al., 2017). This adopts a non-theoretical approach and do not specify a variant based on prior observation (Carter et al., 2017). This include GWAS without the further selection of candidates or studies where a neuroimaging abnormality *per se*

could be assessed as a phenotype for the investigation of the impact of genetic variations on specific structural or functional neuroimaging abnormalities. This later approach may also be used in a hypothesis-free basis to investigate genetic variations that could survive stringent statistical correction for multiple comparisons on specific imaging (endo) phenotypes. Finally, as a disorder or trait may be associated with several genes, there is the more recent *polygenic approach*, in which several variants can be analyzed in the form of a polygenic risk score, or the occurrence of summation or epistatic interactions between different genes can be investigated (Wray et al., 2014).

Although imaging genetics studies have flourished in the literature, concerns regarding their reproducibility have been raised (Carter et al., 2017). For example, a recent systematic review of 40 imaging genomics studies probing the impact of 7 genes that reached genome-wide significance for schizophrenia and bipolar disorder found that most studies were limited by small sample sizes, poor clinical-genetic design, and a lack of statistical correction for multiple comparisons, while few true replications were reported (Gurung and Prata, 2015). Similarly, a recent systematic review that included 44 imaging genetics studies enrolling participants with bipolar disorder found a single replicated finding, namely the association of the BDNF Val66Met 'Met' allele with reduced hippocampal volumes (Pereira et al., 2017).

Several imaging genetics studies involving participants with unipolar depression have been performed (Alexopoulos et al., 2009; Frodl et al., 2008; Frodl et al., 2004; Frodl et al., 2010; Hickie et al., 2007; Kanellopoulos et al., 2011; Taylor et al., 2005; Wang et al., 2012). However, to our knowledge, findings have been inconsistent across studies, with most studies adopting the candidate gene approach. For example, one study found that individuals with MDD who were carriers of the *BDNF* Val66Met 'Met' risk allele had smaller hippocampi than non-carriers (Frodl et al., 2014), while another study failed to replicate this finding (Cole et al., 2011). Similarly, the effects of polymorphisms of the serotonin transporter promoter region (5-

HTTLPR) on hippocampal volumes in individuals with MDD have not been consistent across studies. While some studies suggest that carriers of the *5-HTTLPR* long 'L' allele could exhibit smaller volumes (Taylor et al., 2005), others found no effect of the polymorphism on hippocampal volumes (Ahdidan et al., 2013). Several potential sources of heterogeneity could explain the discrepant findings, including the selection of samples with different characteristics across studies, or different procedures used for image acquisition and analysis. For instance, the correlations between different automated methods for the assessment of amygdala and hippocampal volumes against manual segmentation methods do not appear to correlate strongly (Schoemaker et al., 2016).

The emergence of many imaging genetics studies conducted in participants with MDD in the literature as well as the discrepant findings across studies prompted us to perform a comprehensive systematic review and meta-analysis of imaging genetics studies involving participants with MDD. The identification of replicated evidence from studies that reported the impact of gene variants on both pre-determined regions of interest (ROI) as well as functional and structural magnetic resonance imaging (MRI) studies that provided whole-brain analysis were synthesized. In addition, when replicated evidence was available we estimated the power of the meta-analysis due to concerns of lack of proper statistical power in this specific field (Carter et al., 2017). We considered for inclusion studies that primarily enrolled participants with MDD to identify possible imaging endophenotypes that could have emerged from this literature. However, neuroimaging data that assessed the effects of the same genetic variants in control groups were also extracted whenever available. This approach aimed to identify possible gene versus group (i.e., MDD compared to controls) interactions on brain structure and function. If a particular genetic variant is associated with an imaging finding in MDD subjects, we expected that carriers of the polymorphism would have altered values with respect to the non-carriers, which would not occur in the healthy controls carriers of that particular allele.

Whenever a genetic variant is associated with imaging alterations in either MDD or HCs, it is expected that the effect sizes of the differences with respect to the non-carriers of the allele are larger for the MDD subjects. Furthermore, sources of heterogeneity across studies were explored aiming to shed light on possible factors that could be harmonized in future collaborative efforts to improve the overall reproducibility of this field.

2. Methods

2.1. Search strategy and eligibility criteria

This systematic review and meta-analysis complied with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2010), and followed an *a priori* defined protocol (available upon request to the authors). The EMBASE, PubMed/MEDLINE and PsycINFO electronic databases were searched from inception up to June 30th, 2017 (detailed search strings are provided in the Supplementary Material, available online). The search strategy was augmented through hand searching the reference lists of eligible articles. Two investigators (LPP and BPF) independently screened the tiles/abstracts of retrieved references, assessed full-text for eligibility, extracted data and rated the methodological quality of eligible studies. Disagreement were resolved either through consensus or consultation with a third investigator (CAK or AFC). The following inclusion criteria were applied: (1) human studies that enrolled participants of any age meeting diagnostic criteria for depression (for example, DSM or ICD) regardless of the inclusion of a healthy control group or design (e.g. cross-sectional, case-control or prospective studies were eligible); (2) provided an association of genetic variants and structural or functional MRI measures; and (3) studies that provided either ROI analysis (e.g., mean values of volumes or functional activation, standard deviations and/or statistical difference [p] or [t] between groups) or whole brain results of changes in a stereotactic space in three dimensional coordinates (x, y, z), using significance thresholds either corrected for voxel based multiple comparisons or uncorrected

with spatial extent thresholds. The following exclusion criteria were considered: (1) extractable data were not available; (2) a larger study reporting on the same association and with an overlap of samples was available; (3) investigations that used imaging methods other than structural or functional MRI; (4) studies that enrolled samples with mixed diagnoses unless data for MDD was reported separately; (5) studies that reported solely associations with treatment response or other clinical measures; (6) preclinical or post-mortem studies, case series, literature reviews, conference papers, meeting abstracts or meta-analyses. We included studies regardless of the specific ROI (i.e., brain area) investigated, and there was also no restriction relative to the inclusion of studies that assessed specific gene variants. The overall agreement between the two investigators was adequate (84.9%).

2.2. Data Extraction

Corresponding authors were contacted in at least two occasions when extractable data were not provided in the original report. The following variables were considered for extraction: first author, year of publication, sample size, age of participants, % of females, diagnostic criteria for MDD, number of previous major depressive episodes, depression severity scores, use of psychotropic medications (percentage of the sample that used antidepressants and/or antipsychotics), name of the gene(s) and allele groups assessed, MRI scanner field strength (1.5 T vs. 3.0 T), and details of imaging methods and procedures. For ROI analyses, mean volume or activation within the ROI and the standard deviation (SD) or t and P values with the direction of the change were extracted, as well as ROI tracing methods (i.e., manual vs. automatic). For voxel-wise studies (voxel-based morphometry [VBM] or whole-brain functional MRI analysis [fMRI]), coordinates were extracted according to the ES-SDM method (Radua et al., 2012). Data from control groups were also extracted whenever a study included a comparison group. The agreement between the two investigators was 91.2%.

2.3. Methodological quality appraisal

A previously published 11-item tool (Karg et al., 2011) was used to rate the methodological quality of included studies. The items and quality ratings are provided in Table S8 (available online). This tool is based on criteria of the Strengthening the Reporting of Genetic Association Studies (STREGA) (Little et al., 2009) and the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) (von Elm et al., 2008). It was previously used in a meta-analysis of imaging genetics studies (Molendijk et al., 2012a). An overall quality score was computed as the percentage of criteria met by each study. Agreement among the independent raters was adequate (82.3 %).

2.4. Statistical analysis

2.4.1. Meta-analyses of studies that provided ROI data

A standardized mean difference [Hedges's g; Hedges (1981)] was estimated for each ROI measure (volume or activation) (Lau et al., 1997). A positive value of this metric indicated decreased ROI volumes or functional activity in carriers in comparison to non-carriers of the genetic risk variant, in either participants with MDD or healthy controls (HCs).

Effect size (ES) estimates were pooled using a random-effects model (DerSimonian and Laird, 1986) when at least three individual studies investigated the association. For meta-analyses of total volume or activation, we first estimated study-level ESs for the studies that provided separate measures for the left and right sides. The unilateral ESs were combined to obtain a single ES for the study. These were then pooled together with studies that provided only bilateral measures to obtain the final meta-analytic estimate. Heterogeneity across studies was quantified with the I^2 statistic and assessed using the Cochran's Q test (Borenstein et al., 2009; Patsopoulos et al., 2009).

Publication bias was assessed by inspection of funnel plot asymmetry and through the Egger's test (Egger et al., 1997). Small-study effects (indicative of publication bias) were defined by a P-value < 0.1 in the Egger's test combined with the ES of the largest study more

conservative or in the opposite direction in comparison to the overall ES estimate (Carvalho et al., 2016a; Carvalho et al., 2016b). The trim-and-fill procedure was used to adjust the ES for publication bias when small-study effects were verified (Duval and Tweedie, 2000), while the fail-safe N (i.e., the file drawer statistic) was used to determine how many additional studies would be necessary to turn a significant ES non-significant (Rosenthal, 2017).

We explored potential sources of heterogeneity using either subgroup or random-effects meta-regression analyses (Trikalinos et al., 2010). Meta-regression analyses considered the following potential moderators: sample size, mean age, % of females, methodological quality scores, year of publication, mean depressive symptom scores, mean illness duration in years, and % of participants using psychotropic medications. All analyses were conducted in Stata MP software version 14.0 (Stata Corp, College Station, TX, USA) using the metan package. We estimated the power of each summary ES estimate across a hypothetical range of 'true' ESs with a method developed by Hedges and Pigott (2001). Statistical significance was considered at an alpha level of 0.05.

2.4.2. Meta-analyses of studies that provided whole-brain data

The effect-size version of signed differential mapping (ES-SDM) was used to perform separate voxel-based meta-analyses of regional gray matter volume, diffusion-tensor imaging fractional anisotropy measures or functional brain response abnormalities. ES-SDM is a relatively novel method that incorporates aspects of the activation likelihood estimate (ALE) and multilevel kernel density analysis (MKDA) approaches (Radua and Mataix-Cols, 2009, 2012; Radua et al., 2012). For each study, peak coordinates were used to recreate a map of the ES of the differences between carriers vs. non-carriers of genetic risk variants, and then a standard random-effects variance-weighted meta-analysis was performed for each voxel. Default ES-SDM kernel size and thresholds were used (FWHM = 20 mm, voxel P = 0.005, peak height Z = 1, cluster extent = 10 voxels) (Radua et al., 2012). Meta-analyses were conducted when whole-

brain measures were available for at least two individual datasets for each method. The CARET v5.65 Software was used to visualize SDM results (http://brainmap.wustl.edu/caret.html).

3. Results

3.1. Study selection

The literature search retrieved 2409 records, while an additional reference was found after reviewing the reference lists of included articles. After removal of duplicates, 1499 unique references were screened. One thousand, three hundred and eighty-eight references were excluded after title/abstract screening. Of the 111 full-texts assessed, 46 were excluded with reasons (see Table S1, supplementary online material). Therefore, 65 imaging genetics studies that assessed 31 candidate genes met inclusion criteria for the qualitative systematic review (Figure 1), which provided data from 7327 participants (4034 participants with MDD and 3293 HCs). Finally, 22 studies provided data for quantitative meta-analyses (N=1498; 882 participants with MDD and 616 HCs). See supplementary online results for details of included references.

[Please insert Figure 1 here]

3.2. Characteristics and methodological quality assessment of included studies

Thirty-one of the 65 studies investigated non-replicated associations of candidate gene polymorphisms and imaging findings (Table S2, available online). The remaining 34 studies (22 included in the meta-analyses) reported replicated associations for the *5-HTTLPR* (k=16), *BDNF* Val66Met (k=13) and the *COMT* Val158Met (k=5) polymorphisms (see Tables S3 to S7, available online).

Forty-six studies (70.1%) included a HC comparison group, while the remaining enrolled only participants with MDD. Overall, 1664 participants with MDD (range = 1 to 144) carried genetic variants of risk (RCs), while 1460 (range = 5 to 169) were non-risk carries

(NRCs). For HCs, 921 (range = 2 to 123) were RCs, whilst 1668 (range = 4 to 508) were NRCs.

Across included studies, most participants were females (65.1% in MDD and 57.5% in HC groups, respectively), and the mean age was 45.7 (SD = 11.8) in subjects with MDD and 43.2 (SD = 13.4) among HCs. Medication status was reported in 44 studies (67.7%), of which 12 (27.3%) reported that at least 50% of participants with MDD were currently taking more than one psychotropic medication. Eleven studies (16.9%) reported that MDD samples were drug-free at the time of MRI scan. There was substantial between-study variability in the methodological quality, but it was overall good (median = 89%, range 63–100; Table S8, available online).

Twenty-four studies (36.9%) assessed brain volume using ROIs, of which 15 (62.5%) used manual techniques to segment the tissue volume. The remaining used either semi-automated or automated segmentation methods (4.2% and 33.3%, respectively). Across these studies, the hippocampus was the most extensively investigated brain structure (79.2%), followed by the amygdala (12.5%), and the caudate (8.3%). The putamen, orbitofrontal cortex (OFC), dorsolateral prefrontal cortex and striatum were reported only by single studies. Finally, seven studies (10.7%) used voxel-based-morphometry to assess volume.

Twenty-four studies (36.9%) used functional blood-oxygen level dependent (BOLD) fMRI during tasks. Of these studies, 15 (62.5%) used a ROI for analysis (amygdala [k=10], caudate [k=2], insula [k=1], prefrontal regions [k=3], anterior cingulate cortex [k=3] and parahippocampal region [k=1]), whereas 9 (37.5%) used whole-brain analysis. The sad faces paradigm (k=6), negative pictures (International Affective Picture System - IAPS) (k=3), fearful faces (k=1), emotional processing (k=1), emotional memory (k=1), episodic memory (k=1) and emotional word processing (k=2) were the most frequently used tasks across functional studies. Three studies provided whole-brain resting state fMRI data.

Finally, 9 studies (13.8%) used diffusion-tensor imaging (DTI) to assess brain connectivity. Most of these studies performed whole-brain tractography (volume-based [k=2] and tract-based spatial statistics analyses [k=5]), while 4 studies reported diffusion metrics for ROIs.

The MRI scanner field strength ranged from 1.5T to 3.0T, with the majority using 3.0T (56.9%) scanners. Six studies did not inform the MRI field strength.

3.3. Meta-analyses of studies that provided ROI data

Of the 22 studies included in the meta-analyses, 16 (72.7%) reported ROI metrics (hippocampal volume [k=13] and amygdala function [k=3]). Three studies reported data on both *5-HTTLPR* and *BDNF* Val66Met polymorphisms (Cole et al, 2011; Jaworska et al, 2016; Phillips et al, 2015). Furthermore, 3 studies (Benjamin et al, 2010; Opmeer et al, 2013; Wang et al, 2012) did not provide sufficient information to estimate effect sizes because data for both RCs and NRCs groups were presented for the combined MDD and HC groups (Table S5, online supplement).

[Please insert Table 1 here]

3.3.1. *5-HTTLPR* polymorphism

Hippocampal volume differences associated with the *5-HTTLPR* polymorphism were investigated in 8 studies. Among participants with MDD, 211 were carriers of the *5-HTTLPR* short 'S' risk allele (RCs), and 99 were carriers of the long 'L' non-risk allele (NRCs). Five of the eight studies also provided data for HCs (160 RCs and 70 NRCs). Hippocampal volumes of the participants with MDD or HCs who were carriers of the short 'S' risk allele were not different than carriers of the long 'L' allele (MDD, $g = 0.193$; $P = 0.157$; HC, $g = 0.200$; $P = 0.163$; Table 1 and Fig. 2a; Figure S1, available online). No evidence of small-study effects was observed in the meta-analysis of MDD participants, while there was evidence of this type of bias in the meta-analyses of the HC comparison group for the total or right hippocampal

volumes (Table 1). This effect sizes did not survive correction for publication bias (Total: g = 0.127, 95%CI = -0.133–0.387; right: g = 0.080, 95%CI = -0.180–0.340). No statistically significant differences emerged for the right or left hippocampal volumes for either MDD or HC subjects (Table 1; Figures S2–S5, available online).

Heterogeneity was low in the meta-analyses of hippocampal volumes in MDD subjects (I^2 = 15.5%–30.0%) and inexistent for HCs (Table 1). Meta-regression showed that differences in gender distribution (% females) in the MDD group emerged as a significant moderator, as a higher % of females was associated with a reduced difference in total hippocampal volume between carriers of the *5-HTTLPR* short 'S' allele and non-carriers (Table S9). Subgroup analyses further showed that heterogeneity was higher in studies that employed manual tracing compared to those that used semi-automated or automated segmentation methods (I^2 = 37.1%). In studies that enrolled a younger or predominantly female MDD sample, total hippocampal volume in carriers of the *5-HTTLPR* 'S' allele was reduced when compared to non-carriers (% of females in sample >= 70%, g = 0.635, *P* = 0.006; age < 40 years old, g = 0.635, *P* = 0.016; Table S11). Finally, for MDD subjects that were drug-free during assessment, carriers of the 'S' allele had reduced right hippocampal volume in comparison to carriers of the 'L' allele (g = 0.520, *P* = 0.045; Table S11, available online).

Total amygdala activation was investigated in 3 studies (Table 1). In these studies, the neurofunctional responses to sad faces relative to neutral faces was considered as activation. There was no difference in amygdala activation between carriers of the *5-HTTLPR* 'S' allele (RCs) and carriers of the *5-HTTLPR* 'L' allele (NRCs) in both MDD (g = -0.896; P = 0.145) and HC subjects (g = -0.364; P = 0.613; Table 1; Figures S6 and S7, available online). Heterogeneity was very large (I^2 = 84.8% for MDD and 86.5% for HC).

[Please insert Figure 2 here]

3.3.2. *BDNF* Val66Met polymorphism

Eight studies investigated hippocampal volume differences between carriers of the *BDNF* Val66Met 'Met' risk allele (RCs) and carriers of the *BDNF* Val66Met non-risk allele ('Val'; NRCs). The *BDNF* Val66Met 'Met' allele was not significantly associated with alterations in total hippocampal volume in MDD subjects (g = 0.029; P = 0.832) or HCs (g = 0.221, P = 0.662 Table 1 and Fig. 2b). Moreover, right or left hippocampal volumes in both MDD and HC groups were also similar in carriers of the 'Met' allele in comparison to non-carriers (MDD group: right g = 0.034, P = 0.837; left g = 0.027; P = 0.825; HC group: right g = 0.264, P = 0.331; left g = 0.177, P = 0.291; Table 1 and Figs. S9–S12, available online). No evidence of small-study effects was observed and between-study heterogeneity was large (Table 1), especially for studies that measured the right hippocampus (MDD subjects: I^2 right = 54.6%; I^2 left = 17.2%; HCs: I^2 right: 82.6%, I^2 left: 55.6%).

The methodological quality of included studies emerged as a significant moderator in meta-regression analysis of studies that measured the right hippocampus in the MDD group (Table S9), while publication year was a significant moderator in studies that measured the left hippocampus in the HC group (Table S10). The ES estimate was higher (i.e. indicating smaller hippocampal volumes as a function of the 'Met' allele carrier status) in studies with better methodological quality and lower with increasing publication year, respectively. In addition, subgroup analyses suggested that heterogeneity was absent in studies in MDD subjects that included total brain volume as a covariate (Table S11).

3.4. Meta-analyses of studies that provided whole-brain data

From the 22 studies included in the meta-analyses, 6 reported whole-brain data and were included in the SDM meta-analyses. Two studies used VBM to assess grey matter volume, 2 used DTI to assess brain white-matter differences, and the remaining two used fMRI to investigate brain activation. These studies investigated the *5-HTTLPR* or the *BDNF* Val66Met polymorphism in MDD subjects.

3.4.1. *5-HTTLPR* polymorphism

The role of the *5-HTTLPR* polymorphism in MDD was investigated with whole-brain MRI techniques in 5 studies (k=2 DTI; k=2 fMRI; k=1 VBM; Tables S6 and S7, available online). None of the two voxel-wise functional studies reported statistically significant results (Table S6). The VBM study found a statistically significant reduction in total hippocampal volumes (Frodl et al, 2008). Nevertheless, a small volume correction was applied to this ROI, which prevented its inclusion in a multimodal cortical meta-analysis. Therefore, a SDM meta-analysis was performed including the 2 DTI studies. Various brain regions with statistically significant decreases in white-matter FA in MDD participants carrying the *5-HTTLPR* short 'S' allele compared to non-carriers were verified, with a predominance of voxels centered in the corpus callosum (Fig. 3; Table S14, available online).

[Please insert Figure 3 here]

3.4.2. *BDNF* Val66Met polymorphism

Four studies met criteria for SDM meta-analysis (k=2 fMRI, k=2 VBM; Table S6, available online) and were pooled using a multimodal cortical analysis. Participants with MDD carrying the *BDNF* Val66Met 'Met' risk allele presented conjoint abnormalities with an increase in gray matter volumes accompanied by an hyperactivation or failure of deactivation in the right middle frontal gyrus (orbitofrontal region; Fig. 4; Table S15, available online). One fMRI study used an activation task [emotional appraisal of scenes from the IAPS; Lisiecka et al. (2015)], whereas the other included fMRI study used a resting-state approach (Yin et al., 2015).

[Please insert Figure 4 here]

4. Discussion

To our knowledge, this systematic review and meta-analysis provides the most extensive synthesis to date of studies that investigated the association of putative risk genetic variants

with structural and functional neuroimaging findings in individuals with MDD. All included studies followed a candidate gene approach, while no studies that followed a discovery-based approach (Carter et al., 2017) and that provided extractable data for MDD participants were identified. Although 65 studies were identified, only a few replications could be meta-analyzed. These studies investigated associations related to the *5-HTTLPR* and *BDNF* Val66Met risk polymorphisms. Moreover, our analyses identified possible sources of heterogeneity across studies, which might provide insights for the design of future studies that could enhance the overall reproducibility in the field of imaging genetics in MDD.

We found no evidence for an association of either the *5-HTTLPR* short 'S' allele or the *BDNF* Val66Met 'Met' risk polymorphisms and hippocampal volumes in either participants with MDD or HCs in studies that provided ROI data (see Table 1 and Fig. 2). Also, this finding was confirmed in an exploratory multimodal whole-brain meta-analysis of studies assessing the impact of the *BDNF* Val66Met 'Met' allele on brain structure and function (see Fig. 4) while no replicated evidence for whole-brain structural MRI abnormalities associated with the *5-HTTLPR* risk polymorphism was available. A functional variation in the promoter serotonin transporter gene (also referred to as *SLC6A4*), and in particular the low-expressing short ('S') allele has been implicated in the development of depression following stress exposure (Caspi et al., 2010; Caspi et al., 2003). Nevertheless, a recent collaborative individual-participant meta-analysis provided no evidence for such a gene-environment interaction of the *5-HTTLPR* polymorphism and depression (Culverhouse et al., 2017). Our finding suggests that, if this polymorphism confers a risk for MDD, then its effect could be mediated by a mechanism other than an influence on hippocampal volumes. However, it is worthy to note that meta-regression and subgroup analysis identified several possible sources of heterogeneity that could have influenced these ES estimates. For example, characteristics of samples across studies (i.e., gender) and medication status appeared to moderate these results (see Tables S9 to S12).

Furthermore, heterogeneity was lower in studies that used automated segmentation methods instead of manual or semi-automated ones (see Tables S11 and S12). This finding is consistent with a previous report that verified that even among skillful raters the measurement of hippocampi through manual segmentation methods could be prone to bias (Maltbie et al., 2012).

Several lines of evidence point to a role for BDNF in the pathophysiology of depression. BDNF is the pivotal mediator of the neurotrophin hypothesis of depression, which postulates that MDD is secondary to abnormalities in brain plasticity within brain regions that regulate emotion and memory, including the hippocampus (Duman et al., 1997). Furthermore, preclinical evidence suggests that the therapeutic effects of antidepressants result from the up-regulation of hippocampal BDNF expression (Duman and Monteggia, 2006). Moreover, clinical studies indicate that peripheral levels of BDNF are lower in participants with MDD compared to HCs, and may be influenced by antidepressant treatment (Molendijk et al., 2014). The *BDNF* Val66Met SNP, which represents the substitution of a valine (Val) by a methionine (Met) at codon 66, modifies the sorting of the BDNF protein and its availability in the synaptic cleft. Interestingly Met/Met transgenic mice exhibit a decrease in activity-dependent secretion of BDNF, smaller hippocampal volumes and lower dendritic complexity of hippocampal neurons (Chen et al., 2006; Chen et al., 2004). Our findings are consistent with a previous meta-analysis that also found no evidence of an association of the Val66Met SNP and hippocampal volumes across neuropsychiatric disorders (Harrisberger et al., 2015). Furthermore, MDD is frequently accompanied by cognitive dysfunction encompassing several domains (including declarative memory) that may persist even during symptom remission (Bortolato et al., 2014; Bortolato et al., 2016). Our data also concur with two previous reports that found no significant association of the BDNF Val66Met SNP and emotionally neutral declarative memory retrieval in individuals with MDD (Benjamin et al., 2010; Molendijk et al.,

2012b). Nevertheless, it should be pointed out that there was substantial variability in the methodological quality of included studies, which emerged as a possible source of heterogeneity across studies in meta-regression analyses (see Table S9).

The meta-analysis of whole-brain DTI studies showed that the *5-HTTLPR* polymorphism was associated with widespread microstructural WM abnormalities. There was evidence of decreased FA values, with a predominance in voxels centered in the corpus callosum (CC; see Figure 3). This finding is in accordance with recent meta-analyses of DTI studies. Wise et al. (2016a) showed that MDD is associated with a significant decrease in FA values in the genu of the CC. A separate meta-analysis, conducted in first-episode, medication-naïve participants with MDD, also showed decreased FA in the CC as well as other regions (Chen et al., 2017). Furthermore, lower FA values in the CC was previously reported in adolescents at high family risk for depression compared to controls without a family history of mental disorders (Huang et al., 2011). The corpus callosum is the largest myelinated WM tract in the human brain. It interconnects the cerebral hemispheres to integrate motor, perceptual, and high-level 'hot' and 'cold' (e.g., executive function) cognitive functions. There is evidence that subsets of individuals with MDD have impairment in these cognitive functions (Bortolato et al., 2014; Hofer and Frahm, 2006; Miskowiak and Carvalho, 2014), and we can speculate that the impaired connectivity of the CC may play a role in MDD pathophysiology. Indeed, postmortem and preclinical studies suggest that aberrations in myelination may be involved in the etiology of depression (Tham et al., 2011), while effects of standard antidepressants may at least partly involve pathways governing myelination (Bartzokis, 2012). The current findings suggest that the *5-HTTLPR* polymorphism may underpin these connectivity abnormalities in MDD subjects. However, as only two independent studies were included in our meta-analysis, further well-designed studies are warranted to confirm these preliminary findings.

Previous evidence suggests that the *5-HTTLPR* polymorphism could confer a lower resilience to stress via the modulation of amygdala activation to emotional faces [see Caspi et al. (2010) for a review]. Our meta-analysis provided no evidence of abnormal amygdala reactivity to sad faces compared to neutral faces in participants with MDD who carried the *5-HTTLPR* 'S' polymorphism compared to carriers of the *5-HTTLPR* 'L' polymorphism (see Table 1). However, this finding must be interpreted with caution, as the heterogeneity was large, and only three independent datasets were available. Notwithstanding evidence from postmortem brain studies as well as PET/SPECT studies suggest the expression of the serotonin transporter (5-HTT) is lower in the amygdala of MDD subjects compared to controls, the findings have not been consistent across studies, and heterogeneity is large (Gryglewski et al., 2014; Spies et al., 2015). Therefore, evidence from diverse approaches (ranging from postmortem to imaging genetics studies) are still necessary to clarify the effects of the *5-HTTLPR* polymorphism and the 5-HTT in the amygdala in the pathophysiology of depression.

Multi-modal SDM meta-analysis of 2 VBM studies and 2 fMRI studies observed that, relative to carriers of the non-risk 'Val' allele, MDD participants carrying the BDNF Val66Met 'Met' allele were characterized by conjoint increased activation or failure to deactivate as well as increased GM volumes in a cluster encompassing the right MFG (BA 44; see Fig. 4). A recent multi-modal meta-analysis of VBM studies found a significant reduction in the right MFG in individuals with MDD relative to HCs (Wise et al., 2016b). This finding has also been replicated in a meta-analysis that included only studies involving first-episode MDD participants (Zhang et al., 2016). However, our counterintuitive finding should be regarded as exploratory as only two VBM studies were included, and thus we could not verify the robustness of our findings (for example, through jack-knife sensitivity analysis in which one study is removed per iteration). Furthermore, one of the VBM studies, which reported higher right MFG volumes, enrolled only participants with melancholic depression (Cardoner et al.,

2013). Therefore, this finding may not be extrapolated to the broad depression phenotype. However, it is noteworthy that hyperactivation or failure to deactivate the right MFG has been implicated in the neurobiology of depression. For example, a recent study provided evidence that this functional abnormality could be related to autobiographical problem-solved deficits associated with rumination in depression (Jones et al., 2017). Along similar lines, the right MFG is part of the default mode network (DMN), and failure to deactivate the DMN has been implicated in cognitive control deficits in depression (Jacobs et al., 2014; Pizzagalli, 2011; Rodriguez-Cano et al., 2017). But as only two fMRI studies were included, which employed either resting-state conditions or used an emotional cognitive task, these findings are also limited and should be taken as preliminary.

4.1. Strengths and limitations

The main strength of our work is the inclusion of a large body of data, and the use of state-of-the-art statistical methods. These provide a quantitative appraisal of the available evidence and also allow the identification of potential sources of heterogeneity across studies.

However, some limitations deserve mention. First, few replicated findings were identified. Second, our power analysis suggests that most meta-analytic estimates reported herein are underpowered. Therefore, findings should be interpreted with caution, with the exception of the ES estimates on the association of the *5-HTTLPR* 'S' allele with total hippocampus volume in participants with MDD or HCs, and the association of the *BDNF* Val66Met 'Met' allele with left hippocampal volume in participants with MDD, which were adequately powered (see Table S13).

This review also identified potential sources of heterogeneity across studies that could have influenced the findings. However, depression is a multifaceted and heterogeneous phenotype, and thus the observed heterogeneity could reflect 'true' heterogeneity. Moreover, other possible sources of heterogeneity could not be explored due to lack of available data. An

example is the frequent association of MDD with obesity and metabolic abnormalities (de Melo et al., 2017; Liu et al., 2014). It has been proposed that these co-occurring conditions could impact neuroimaging findings in depression (Cha et al., 2014; Gupta et al., 2015).

The literature on either structural or functional neuroimaging data is limited by an excess of reporting biases (Fusar-Poli et al., 2014; Ioannidis, 2011). Indeed, a recent large-scale whole-brain meta-analysis of functional neuroimaging studies suggested a lack of consistency and reproducibility across studies conducted in MDD samples (Muller et al., 2017). Our findings could be affected by those biases as well. Additionally, the studies included in our review used a candidate gene approach. As the potential methodological strengths of exploratory genome-wide approaches are being increasingly recognized (Carter et al., 2017), an unbiased assessment is less likely with the methodology employed in the studies identified by our review. Furthermore, although a validated tool for the appraisal of the methodological quality of gene-imaging studies is currently unavailable, we found substantial between-study variation in the methodological quality of included studies (see Table S8), which was also a source of heterogeneity (see Table S9). Therefore, these variations in study methodology limit the interpretation of the findings.

Finally, it is worthy to note very few studies have separated carriers of two risk alleles (i.e., homozygotes) from participants carrying a single allele of risk. Therefore, a putative dose-response effect of risk alleles cannot be clearly established.

4.2. Research implications

We observed that both sample characteristics across studies (e.g., gender or medications) as well as methodological aspects, moderated some of the ES estimates reported here (see Tables S9–S11). Therefore, future studies should control the potential clinical and sociodemographic variables more precisely. It is important to mention that genetic ancestry was not considered in the studies included in this review, and the ethnic composition of the sample was frequently

missing, which did not allow specific subgroup analyses (see Table S8 which presents the methodological assessment). Futures studies should consider also genotyping ancestry-related SNPs together with the investigated genes to avoid spurious associations with the psychiatric or imaging phenotype.

Furthermore, an improvement in the transparency of protocols (e.g., through *a priori* publication of study procedures), as well as a better harmonization of neuroimaging procedures across centers are warranted. Large imaging genetics collaborative consortia, like ENIGMA, point that the inclusion of large samples with proper methodological harmonization across studies may improve the reproducibility of the field (Stein et al., 2012).

In this systematic review, very few candidate genes had been previously shown to have genome-wide significance for MDD (e.g., *SLC6A15*) (Choi et al., 2016; Hyde et al., 2016). Furthermore, the field still lacks replicated evidence for genes that are relevant to other pathways that may also be involved in MDD. For example, few imaging genetics studies have addressed the relevance of cytokine/chemokine (Kohler et al., 2017) as well as renin-angiotensin (Vian et al., 2017) genes. Therefore, the recent identification of genome-wide significant risk variants associated with MDD open perspectives for imaging genetics studies. The testing of genetic variants supported by GWAS studies or by convergent sources of experimental and clinical evidence could enhance the robustness of findings in this field (Carter et al., 2017). Another innovative research avenue in imaging genetics studies is the assessment of polygenic risk scores on brain structure and function, although there are also concerns of the lack of genotype-phenotypic cross-trait convergence (Bogdan et al., 2017). Lastly, future efforts with a systematic collection of participant-level data may be able to formally test for interaction effects (for example, between group and genotypes) to provide incremental evidence to the stratified analysis herein conducted.

4.3. Conclusion

This systematic review and meta-analysis indicates that imaging genetics studies in MDD have expanded at a rapid pace. However, few replicated findings involving the *5-HTTLPR* and *BDNF* Val66Met polymorphism could be meta-analyzed. The *5-HTTLPR* 'S' allele was associated with WM microstructural abnormalities mainly in the corpus callosum, while the *BDNF* Val66Met 'Met' allele was associated with a higher GM volume as well as hyperactivation or failure to deactivate the right MFG. Although promising, these findings require replication in well-designed studies with adequate statistical power. An improvement in transparency, as well as methodological refinements in imaging genetics studies, may provide more robust and replicable findings within the context of precision psychiatry (Vian et al., 2017). Furthermore, the adoption of trans-diagnostic approaches [such as the US NIMH RDoC framework; Cuthbert and Insel (2013)] could provide novel biologically validated constructs to be tested in imaging genetics studies, and also increase the statistical power of studies, which this meta-analysis found to be low.

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Contributors

LPP, CAK, and AFC designed the protocol and searched the literature. LPP and BPF screened the studies and extracted the data. LPP, CAK, and AFC analyzed the data. LPP, CAK, and AFC wrote the first draft of the manuscript. BS, KWM, GM, BPF, TT, BSF, ARB, MM, and DAP contributed to the interpretation and discussion of the findings, and to the writing of the manuscript. All authors have read and approved the final version of this manuscript for submission.

Conflict of interest

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FIGURE LEGENDS:

Fig. 1. PRISMA flowchart of study selection process.

Fig. 2. Forest plots of studies that assessed differences in total hippocampal volumes in participants with MDD carrying genetic risk variants (RCs) versus non- carriers (NRCs) of (a) *5-HTTLPR* and (b) *BDNF* Val^{66}Met polymorphisms. For the *5-HTTLPR*, RCs are carriers of the short 'S' allele, and for the *BDNF* Val66Met, RCs are carriers of the Met allele. Effect sizes (ESs) are synthesized as Hedges's g (and 95% CIs) under random-effects modelling. Squares depict ES of individual studies (dimension is proportional to study weight), and diamonds represent summary ES estimates. A positive ES represent lower volumes in RCs relative to NRCs. * The Frodl, 2004 and Hickie, 2007 studies reported measures of the right and left hippocampi combined, while the other studies reported separate measures for the right or left sides. In these studies, the right and left values were first combined within the study to provide effect sizes.

Fig. 3. Anatomical distribution of diffusion tensor imaging (DTI) fractional anisotropy (FA) changes in MDD subjects carrying the *5-HTTLPR* short 'S' risk-allele vs. non-carriers of the risk allele (long 'L' allele). Areas with reduced FA in the risk carriers include the corpus callosum, the left superior longitudinal fasciculus III and right cortico-spinal projections. The FA map is superimposed on a high-resolution T1-weighted template with skeletons for the brain tracts. Data are shown going from the right hemisphere to the left hemisphere. The skeletons represented in each small image are: 1, Right cortico-spinal projections; 2, Right thalamus; 3, Brain-stem; 4, Left cortico-spinal projections; 5-7, Left inferior fronto-occipital fasciculus/optic radiations; 8, Left superior longitudinal fasciculus. Abbreviations: MDD, Major depressive disorder; 5-HTTLPR, Serotonin transporter-linked promoter region.

Fig 4. Increases in gray matter with hyperactivation (or failure to deactivate) in the right middle frontal gyrus among MDD participants carrying the *BDNF* Val66Met 'Met' risk-allele vs non-carriers ('Val' allele). Abbreviations: MDD, Major depressive disorder; BDNF, brain-derived neurotrophic factor.

Table 1. Primary meta-analyses of studies measuring region-of-interest volume or activation in carriers vs. non-carriers of *5-HTTLPR* or *BDNF* Val66Met polymorphisms in participants with MDD or healthy controls. For the *5-HTTLPR*, RCs are carriers of the short 'S' allele, and for the *BDNF* Val66Met, RCs are carriers of the 'Met' allele.

Gene and region of interest	*N* studies	*N* RC	*N* NRC	ES (95% CI)	*P*-value (overall)*	*I²*	*P*-value (Egger) †	Small-study effects ‡	Fail-safe N	Adjusted ES (95% CI)§
MDD										
5-HTTLPR Total hippocampus volume	8	211	99	0.193 (-0.074 – 0.460)	0.157	155.5	0.321	N	0	0.193 (-0.074 – 0.460)
5-HTTLPR Right hippocampus volume	6	157	68	0.318 (-0.040 – 0.676)	0.082	277.9	0.424	N	1	0.318 (-0.040 – 0.676)
5-HTTLPR Left hippocampus volume	6	157	68	0.269 (-0.095 – 0.633)	0.147	300.0	0.447	N	0	0.269 (-0.095 – 0.633)
BDNF Total hippocampus volume	8	142	244	0.029 (-0.243 – 0.302)	0.832	355.6	0.348	N	0	0.029 (-0.243 – 0.302)
BDNF Right hippocampus volume	8	142	244	0.034 (-0.293 – 0.361)	0.837	544.6	0.505	N	0	0.034 (-0.293 – 0.361)
BDNF Left hippocampus volume	8	142	244	0.027 (-0.209 – 0.263)	0.825	172.2	0.214	N	0	0.027 (-0.209 – 0.263)
5-HTTLPR Total amygdala function	3	81	33	-0.896 (-2.099 – 0.308)	0.145	848.8	0.377	N	6	-0.896 (-2.099 – 0.308)
Healthy Controls										
5-HTTLPR Total hippocampus volume	5	160	70	0.200 (-0.081 – 0.482)	0.163	0	**0.035**	Y	0	0.127 (-0.133 – 0.387)
5-HTTLPR Right hippocampus volume	4	152	62	0.157 (-0.137 – 0.452)	0.296	0.0	**0.079**	Y	0	0.080 (-0.180 – 0.340)
5-HTTLPR Left	4	15	62	0.146 (-0.149 –	0.332	0.	0.100	N	0	0.064 (-0.197 –

hippocampus volume		2		0.440)		0				0.324)
BDNF Total hippocampus volume	6	1 3 6	2 4 0	0.221 (-0.207 – 0.649)	0.311	7 3 . 4	0.662	N	0	0.221 (-0.207 – 0.649)
BDNF Right hippocampus volume	6	1 3 6	2 4 0	0.264 (-0.269 – 0.796)	0.331	8 2 . 6	0.609	N	1	0.264 (-0.269 – 0.796)
BDNF Left hippocampus volume	6	1 3 6	2 4 0	0.177 (-0.151 – 0.504)	0.291	5 5 . 6	0.734	N	0	0.177 (-0.151 – 0.504)
5-HTTLPR Total amygdala function	3	5 4	2 3	-0.364 (-1.775 – 1.047)	0.613	8 6 . 5	0.670	N	0	-0.364 (-1.775 – 1.047)

Abbreviations: CI, confidence interval; **ES**, effect size; **5-HTTLPR**, Serotonin transporter-linked promoter region; **BDNF**, brain-derived neurotrophic factor; **MDD**, major depressive disorder; **RC**, risk-allele carriers; **NRC**, non-risk carriers; **Y**, Yes; **N**, No; **NA**, not available due to the small number of studies; statistically significant results are in bold.

[*] In Z-test of overall effect.

[†] In Egger's test of publication bias.

[‡] P < 0.1 in Egger's test of publication bias and effect size of the largest study more conservative than the overall effect size or in the opposite direction.

[§] Adjusted using Duval and Tweedie's trim-and-fill procedure.

Highlights

- Genetic-neuroimaging paradigms may provide neurobiological insights to heterogeneous phenotypes like depression;
- A systematic review and meta-analysis of gene-imaging studies involving samples with depression was performed;
- Sixty-five studies (N=4034 participants with depression) met inclusion criteria;
- Carriers of the BDNF Val66Met risk allele may have hyperactivation and elevated gray matter volumes of the right middle frontal gyrus;
- Exploratory voxel-wise meta-analysis suggests that carriers of the 5-HTTLPR risk allele may have white matter microstructural abnormalities compared to non-carriers
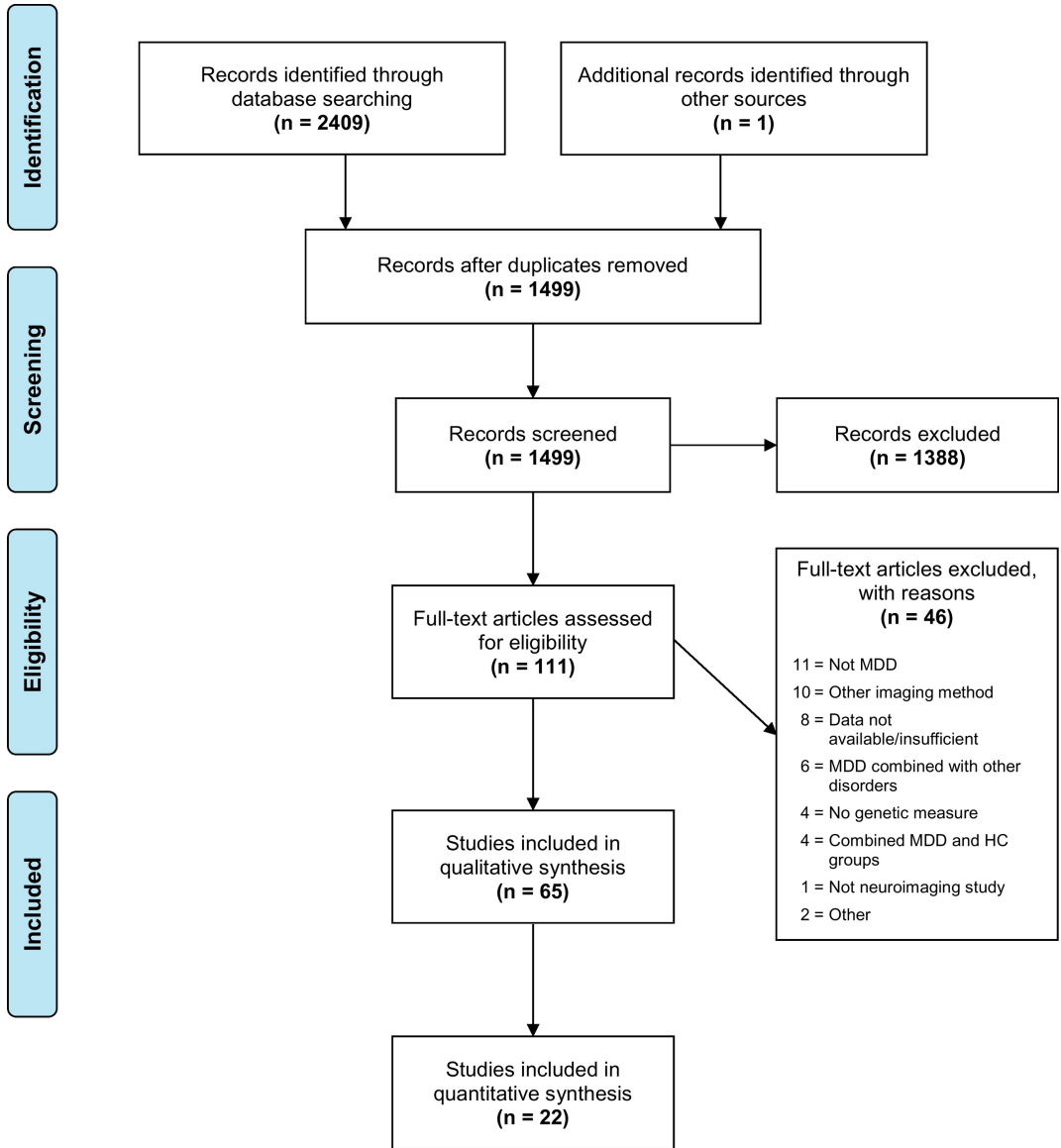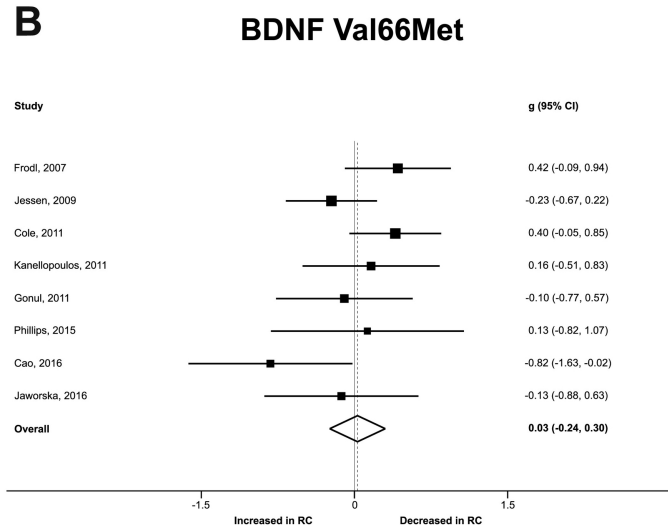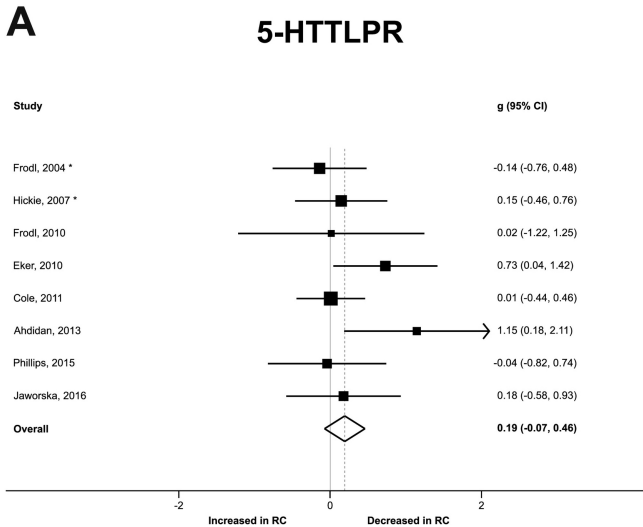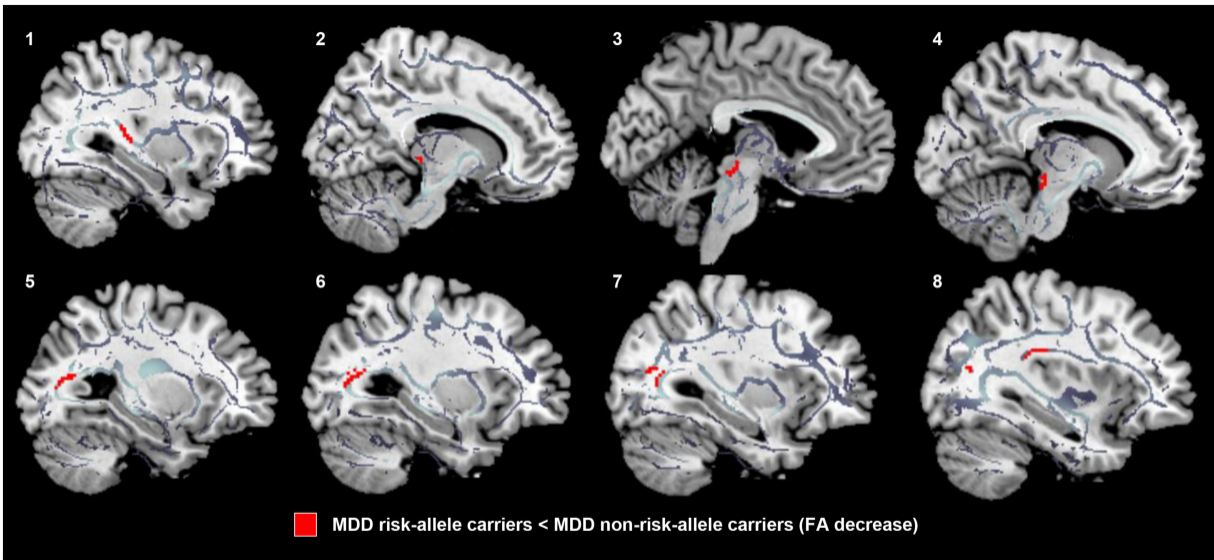
Figure 1

A 5-HTTLPR

Study		g (95% CI)
Frodl, 2004 *		-0.14 (-0.76, 0.48)
Hickie, 2007 *		0.15 (-0.46, 0.76)
Frodl, 2010		0.02 (-1.22, 1.25)
Eker, 2010		0.73 (0.04, 1.42)
Cole, 2011		0.01 (-0.44, 0.46)
Ahdidan, 2013		1.15 (0.18, 2.11)
Phillips, 2015		-0.04 (-0.82, 0.74)
Jaworska, 2016		0.18 (-0.58, 0.93)
Overall		0.19 (-0.07, 0.46)

Increased in RC   Decreased in RC

B BDNF Val66Met

Study		g (95% CI)
Frodl, 2007		0.42 (-0.09, 0.94)
Jessen, 2009		-0.23 (-0.67, 0.22)
Cole, 2011		0.40 (-0.05, 0.85)
Kanellopoulos, 2011		0.16 (-0.51, 0.83)
Gonul, 2011		-0.10 (-0.77, 0.57)
Phillips, 2015		0.13 (-0.82, 1.07)
Cao, 2016		-0.82 (-1.63, -0.02)
Jaworska, 2016		-0.13 (-0.88, 0.63)
Overall		0.03 (-0.24, 0.30)

Increased in RC   Decreased in RC

Figure 2

MDD risk-allele carriers < MDD non-risk-allele carriers (FA decrease)

Figure 3

■ Increase of gray matter volume with hyperactivation

Figure 4