The P2RX7 polymorphism rs2230912 is associated with depression: A meta-analysis

Running title: meta-analysis of P2RX7 in depression

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Conflict of interest:

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Various studies have investigated whether single nucleotide polymorphisms (SNPs) in the gene purinergic receptor P2X7 (*P2RX7)*, and rs2230912 specifically, were associated with mood disorders. While some studies found positive evidence, a large number of studies reported no significant associations. In a previously published meta-analysis, Feng et al. did not find a significant association and only moderate odds ratios (ORs) in case-control studies. They reported significant findings only for family-based studies. We revisited this finding and conducted a meta-analysis including 8,652 cases and 11,153 controls, adding unpublished results from the Munich Antidepressant Response Signature (MARS) study. We found a significant association between rs2230912 and combined mood disorders (major depressive disorder (MDD) or bipolar disorder (BD)) for the allelic, dominant and heterozygous-

disadvantage model, all withstanding the threshold of correction for multiple testing. Stratifying by disorder revealed significant findings for the MDD-subgroup (OR of 1.12 for the allelic model), while the BD-subgroup presented with a lower effect size (OR of 1.05) and no significance. *P2RX7* encodes a purinergic receptor which is expressed in the brain and also localized in immune cells.

Animal studies and functional studies will be necessary to enlighten its involvement in the etiology of mood disorders and its applicability for pharmacological purposes.

keywords: P2RX7, mood disorders, meta-analysis

Introduction

MDD and BD belong to the most common psychiatric diseases with a life-time prevalence of 16% for MDD (Blazer et al. 1994) and 1-3.3% for BD (Jacobi et al. 2004). It is estimated that about 40% of the risk for developing MDD is due to genetic factors (Sullivan et al. 2000), for BD the estimates range from 80 to 90% (Kelsoe 2003).

Linkage studies on BD (Curtis et al. 2003; Dawson et al. 1995; Ewald et al. 1998; Morissette et al. 1999; Shink et al. 2005) have identified a region of interest on 12q24 which was also reported to be involved in linkage for unipolar depression (Abkevich et al. 2003; McGuffin et al. 2005).

P2X, ligand-gated ion channel 7 (*P2RX7*), maps to this region and variations in the *P2RX7* gene have been investigated regarding their role in conferring susceptibility for affective disorders. Since the first report of an association with BD in a French Canadian family-based sample by Barden et al. (Barden et al. 2006) ten subsequent studies have been published testing associations of SNPs in *P2RX7* and BD and/or MDD. Most studies considered exclusively the non-synonymous SNP rs2230912:A>G, which has been identified as the highest associated SNP in the first two published studies (Barden et al. 2006; Lucae et al.

2006). Rs2230912 is located in exon 13 and leads to a change of the amino acid glutamine to arginine at position 460 (Gln460Arg). While three studies reported association of this SNP (Lucae et al. 2006; McQuillin et al. 2009; Soronen et al. 2011), other studies did not detect significant associations with regard to case-control status (Backlund et al. 2012; Green et al. 2009; Grigoroiu-Serbanescu et al. 2009; Halmai et al. 2013; Hejjas et al. 2009; Lavebratt et al. 2010; Viikki et al. 2011; Yosifova et al. 2009).

P2RX7 encodes a purinergic receptor which is involved in Ca2+ dependent signal pathways. It is expressed in the brain and may regulate immune function and neurotransmitter release (Deuchars et al. 2001; Sperlagh et al. 2006). The activation of the receptor provides an inflammatory stimulus and regulates the release of pro-inflammatory cytokines. It could therefore regulate the link between nervous system and immune system (Skaper et al. 2010). Bennett (Bennett 2007) proposed that SNPs in P2RX7 modify the release of cytokines which could change the functional state of neural networks, and may lead to higher vulnerability for mood disorders.

Feng and colleagues (Feng et al. 2014) published a meta-analysis and did not find significant association of rs2230912 with mood disorders in case-control samples. As we wanted to revise these findings and to further enlighten the controversial association reports concerning *P2RX7*, and rs2230912 in special, we conducted a meta-analysis taking into account the published case-control studies and adding results from an in-house sample (MARS study) which have not been published before.

We included all published studies investigating association of rs2230912 with MDD as well as BD because there is a strong phenotypic overlap between both groups of patients. While both MDD and BD patients suffer from depressive episodes, bipolar patients in addition experience manic episodes. Furthermore, a constant lifetime diagnostic conversion rate of 1.25 % per year from MDD to BD has been reported (Angst et al. 2005). However, the

genetic relationship of both disorders remains to be elucidated. Our results may help to further evaluate the potential role of *P2RX7* in mood disorders.

Material and Methods

MARS Study

Unipolar depressive inpatients (demographic information in Table1) were recruited for the Munich Antidepressant Response Signature (MARS) project at the Max Planck Institute of Psychiatry in Munich, Germany (Hennings et al. 2009). Briefly, patients were included in the study within 1–3 days of admission to the hospital and diagnosis was ascertained according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. Patients fulfilling the criteria for at least a moderate depressive episode (HAM-D ≥ 14 on the 21-item Hamilton Depression Rating Scale) entered the analysis. Patients suffered from a first depressive episode or from a recurrent depressive disorder. All included patients were of European descent.

Control subjects were matched to the patient sample for age, gender and ethnicity from a randomly selected Munich-based community sample and underwent a strict screening-procedure for the absence of psychiatric and severe somatic disease (Heck et al. 2009). The overall inclusion rate of all contacted probands was 50.3 %. These subjects thus represent a group of individuals from the general population who has never been mentally ill.

This study has been approved by the ethics committee of the Ludwig-Maximilians-University in Munich and written informed consent was obtained from all subjects.

Genotyping of the MARS study and quality control

On enrolment in the study, EDTA blood was drawn from each patient and control subject and DNA was extracted with the Puregene whole blood DNA-extraction kit (Gentra Systems Inc; MN) from fresh blood using standard DNA extraction procedures. SNP genotyping for

patients and controls was performed on Illumina 317k and 610k Genotyping BeadChips (Illumina Inc., San Diego, USA) according to the manufacturer's standard protocols. The average call rate achieved was higher than 99%, with samples below 98% being either retyped or excluded from the study. SNPs with a callrate below 98%, a deviation from Hardy-Weinberg-Equilibrium (HWE) with a p-value $< 1x10^{-05}$ or with a minor allele frequency below 5% were excluded. For statistical analysis presented in this paper, genotypes for rs2230912 were extracted. These were in HWE for cases (p=0.41) as well as in controls (p=0.40).

Search Strategy

We searched the PubMed database using the keywords "P2X7", "P2RX7", "depression", "bipolar disorder" and "mood disorders". All studies investigating the case-control association between rs2230912 and depression which were included in the database up to Jan 1st 2017 were taken into the analysis. We also considered references from identified articles. In addition, we included an in-house sample (MARS study) for which results had not been published before.

An overview of included studies is given in Table 2. The respective flow diagram is depicted in Figure 1.

Statistical Analysis

Genotypic distributions for BD/MDD cases and controls were derived from the respective publications. If these were not given in the paper, we contacted the authors and asked for more detailed information. This was successful for all but one study (Yosifova et al. 2009). Odds ratios, standard errors and p-values were calculated on the respective 2x2 tables using R (http://www.r-project.org/). P-values were derived from the Wald-test using the R function

oddsratio. We tested three genetic models: allelic, dominant and hethom (i.e. heterozygous disadvantage model).

Meta-analyses for all three models were applied using the Metasoft-Package (http://genetics.cs.ucla.edu/meta/). As there was evidence for heterogeneity of effects between the studies (heterogeneity p-value= 0.015 allelic model, p=0.021 dominant model, p=0.044 hethom model), we applied a random effects meta-analysis using the method of Han and Eskin (Han and Eskin 2011).

Bonferroni's method was applied to correct for the three tested models resulting in a p-value threshold of 0.017 (=0.05/3).

Funnel plots and tests for asymmetry were conducted using the R-library metafor (http://cran.r-project.org/web/packages/metafor/index.html), forest plots were created with the R-library rmeta (http://cran.r-project.org/web/packages/rmeta/index.html).

Power analysis was conducted based on 1,000 simulations. For these, we applied the formulas given in Han and Eskin (Han and Eskin 2011). In each run, we simulated genotype distributions for each study using the study-specific odds ratio, allele-frequency and case and control sample sizes. Afterwards, we calculated effect-size estimates and standard errors for each simulated dataset and ran a meta-analysis over all simulated sets. The power was calculated as the fraction of simulated meta-analysis p-values that was lower than the p-value threshold of 0.017.

Results

We excluded the study of Nagy et al. (Nagy et al. 2008) as this was not based on a case-control comparison. Additionally we removed the study of Yosifova et al. (Yosifova et al. 2009) as no effect size estimates were available here. Effect size estimates for the remaining studies as well as meta-analysis results are given in Table 3. The meta-analysis revealed associations for the allelic model, dominant and hethom model, all withstanding the threshold

of correction for multiple testing (p=0.011 allelic model, p=0.008 dominant model, p=0.009 hethom model). Forest plots and funnel plots for all three models are depicted in Figure 2. Inspection of funnel plots and Egger's test for asymmetry yielded no evidence for publication bias (p=0.206 allelic model, p=0.161 dominant model, p=0.286 hethom model).

In the meta-analysis, we had a power of 89% to detect associations between rs2230912 and mood-disorders withstanding correction for multiple testing.

Stratifying by disorder was possible for all studies except for Lavebratt et al. and Hejjas et al. where no disease-specific individual genotype distributions nor effect size estimates were given. In the meta-analysis of 4,182 MDD cases versus 5,926 controls (including the studies of Lucae et al., Green et al., Grigoroiu-Serbanescu et al. –German sample, Soronen et al., Vikii et al., Halmai et al. and the MARS study), all models were nominal significant (p=0.002 allelic, p=0.037 dominant, p=0.028 hethom model), however only the allelic model withstood correction for multiple testing.

For BD only, the total sample size was 3,813 cases versus 6,691 controls (Barden et al., Green et al., McQuillin et al., Grigoroiu-Serbanescu et al. , Soronen et al., Halmai et al., Backlund et al.). This resulted in no significant p-values in the meta-analysis (p=0.136, allelic, p=0.135, dominant, p=0.271 hethom model). Effect sizes for MDD were higher (OR of 1.12 for the allelic model in the meta-analysis) as compared to BP (OR of 1.05 for the same model). As most of the studies looked at a combined MDD/BP case sample in comparison to controls, separation of controls was not possible. Hence, most of the controls overlap between the stratified MDD and BD meta-analysis.

Discussion

In this meta-analysis we revisit the controversial association findings which have been reported for rs2230912 and mood disorders.

Although Feng et al. (Feng et al. 2014) reported lack of association in their meta-analysis in case-control samples, we found that rs2230912 was significantly associated with MDD/BD. Our study was well-powered, our result withstood correction for multiple testing and we did not find any evidence for publication bias.

This contradictory result could be explained by the fact that the set of studies included in our analysis differs from the studies included by Feng and colleagues. They excluded the results from Lucae et al. (Lucae et al. 2006) due to deviation from Hardy-Weinberg-Equilibrium (HWE). Different reasons could be responsible for this deviation. First, this could be due to genotyping error. However, as Lucae et al. mention in their publication, they re-genotyped rs2230912 using a different genotyping technique and could confirm the genotypic distribution. Second, if heterozygotes represented with the high-risk genotype and if controls were screened for mental disorders, a lack of heterozygotes in the control group can be expected. In that case, the control group would not be in the HWE. This is also in line with the study of Aprile-Garcia et al. (Aprile-Garcia et al. 2016) who showed that the heterozygous state of the SNP impairs receptor function with respect to calcium influx, channel currents and intracellular signaling and hence this state is probably the risk state. Furthermore, rs2230912 is just slightly out of HWE (p=0.03), and this p-value would not withstand correction for multiple testing over all included studies, we therefore decided to leave the results from Lucae et al. in our analysis. To clarify if our meta-analysis was mainly driven by the study of Lucae et al., we also ran an analysis after exclusion of the Lucae study. The allelic model was still nominally significant (p=0.031) while other models were not (p=0.089 dominant model, p=0.191 hethom model). The result for the allelic model did not survive multiple testing correction. However, one should take into account that this study was also one of the largest one with over 2,000 individuals. The fact that we detected only nominal significance could hence also be due to the reduced sample size.

No individual genotype counts nor effect size estimates were available for the study by Yosifova and colleagues (Yosifova et al. 2009) to us, therefore we had to exclude these findings from our analysis.

In comparison to Feng et al. we added a new study (Halmai et al. 2013) to the analysis as well as a new dataset for which results on rs2230912 have not been published before. Feng et al. excluded the study of Backlund et al. due to "duplicate report". However we checked with the authors of the studies published by Backlund et al. and Lavebratt et al. and they ensured that samples for the case-control analyses did not overlap. Therefore we kept both studies in the meta-analysis.

A possible critique of our study might be that we included studies on major depression as well as on bipolar disorder. We were interested whether rs2230912 was associated with mood disorders in general. Furthermore, there is a strong phenotypic overlap between both groups of patients. For those studies, where stratifying into MDD and BP was possible, we additionally ran separate meta-analyses. For the MDD sub-cohort the allelic model withstood correction for multiple testing, while no model was significantly associated for the BD sub-cohort. While we found comparable effect sizes for the combined MDD/BP sample as Feng et al., our findings with regard to stratification differ. While Feng et. al report an OR of 1.01 for MDD only and of 1.08 for BP only, we observed larger effect sizes for the MDD-cohorts (OR=1.14 for the allelic model) as compared to the BP cohort (OR=1.05 for the allelic model).

We focused on one single SNP in our meta-analysis. A broader approach involving more SNPs and ideally tagging all variation of the *P2RX7* locus will be necessary to determine to what extent *P2RX7* itself is involved in mood disorders.

Another important point needs to be addressed. While some cohorts (Lucae et al., Hejjas et al., McQuillin et al., Grigoroiu et al. – Romanian subsample, Lavebratt et al., Soronen et al., Viiki et al., Halmai et al., MARS study) used screened controls, all other studies included in

our meta-analysis included unscreened controls. With the prevalence of 16% for MDD and of 1% for BP in the general population, we cannot exclude that some of these controls were in fact suffering from mood disorders. If we restricted our meta-analysis to the cohorts with screened controls only, in general our effect size estimates increased and the meta-analysis p-values decreased. Future studies should therefore focus on screened controls, otherwise results might be biased.

Furthermore, we included studies which explicitly investigated if P2RX7, and rs2230912 specifically, was associated with mood disorders. Large genome-wide scale consortial studies of course also implicitly checked for association with this SNP. However, using the public available data from the Psychiatric Genomics Consortium (Major Depressive Disorder Working Group of the Psychiatric et al. 2013; Psychiatric 2011) whose MDD cohort also contains the sample of Lucae et al and the MARS study, rs2230912 was not significantly associated with neither MDD nor BP. The PGC is one of the largest studies to date that examined genome-wide associations with MDD or BP. Together with the great benefit of the very large sample-size, the cohorts are also very heterogenous and mostly used unscreened population controls and often non-clinical cases. Effects of rs2230912 might only present in more homogenous samples with regard to disease status, disease severity and screening.

Taking all our findings together and considering that a minor change in included studies led to different results in the meta-analysis, it seems evident that more studies are necessary to determine if rs2230912 is associated with mood disorders. Ideally these studies should be recruited as homogenously as possible. In future, the inclusion of larger studies may also allow to look more closely into whether our findings with regard to higher effect-sizes for MDD as compared to BP can be replicated and also to stratify by sex or different ethnicities. Furthermore, more focused studies on the SNP-effect in animal models could help to enlighten which genetic model exactly describes the association in the best way.

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This study has been approved by the ethics committee of the Ludwig-Maximilians-University in Munich and written informed consent was obtained from all subjects.

Genotyping of the MARS study and quality control

On enrolment in the study, EDTA blood was drawn from each patient and control subject and DNA was extracted with the Puregene whole blood DNA-extraction kit (Gentra Systems Inc; MN) from fresh blood using standard DNA extraction procedures. SNP genotyping for

patients and controls was performed on Illumina 317k and 610k Genotyping BeadChips (Illumina Inc., San Diego, USA) according to the manufacturer's standard protocols. The average call rate achieved was higher than 99%, with samples below 98% being either retyped or excluded from the study. SNPs with a callrate below 98%, a deviation from Hardy-Weinberg-Equilibrium (HWE) with a p-value $< 1x10^{-05}$ or with a minor allele frequency below 5% were excluded. For statistical analysis presented in this paper, genotypes for rs2230912 were extracted. These were in HWE for cases (p=0.41) as well as in controls (p=0.40).

Search Strategy

We searched the PubMed database using the keywords "P2X7", "P2RX7", "depression", "bipolar disorder" and "mood disorders". All studies investigating the case-control association between rs2230912 and depression which were included in the database up to Jan 1st 2017 were taken into the analysis. We also considered references from identified articles. In addition, we included an in-house sample (MARS study) for which results had not been published before.

An overview of included studies is given in Table 2. The respective flow diagram is depicted in Figure 1.

Statistical Analysis

Genotypic distributions for BD/MDD cases and controls were derived from the respective publications. If these were not given in the paper, we contacted the authors and asked for more detailed information. This was successful for all but one study (Yosifova et al. 2009). Odds ratios, standard errors and p-values were calculated on the respective 2x2 tables using R (http://www.r-project.org/). P-values were derived from the Wald-test using the R function

oddsratio. We tested three genetic models: allelic, dominant and hethom (i.e. heterozygous disadvantage model).

Meta-analyses for all three models were applied using the Metasoft-Package (http://genetics.cs.ucla.edu/meta/). As there was evidence for heterogeneity of effects between the studies (heterogeneity p-value= 0.015 allelic model, p=0.021 dominant model, p=0.044 hethom model), we applied a random effects meta-analysis using the method of Han and Eskin (Han and Eskin 2011).

Bonferroni's method was applied to correct for the three tested models resulting in a p-value threshold of 0.017 (=0.05/3).

Funnel plots and tests for asymmetry were conducted using the R-library metafor (http://cran.r-project.org/web/packages/metafor/index.html), forest plots were created with the R-library rmeta (http://cran.r-project.org/web/packages/rmeta/index.html).

Power analysis was conducted based on 1,000 simulations. For these, we applied the formulas given in Han and Eskin (Han and Eskin 2011). In each run, we simulated genotype distributions for each study using the study-specific odds ratio, allele-frequency and case and control sample sizes. Afterwards, we calculated effect-size estimates and standard errors for each simulated dataset and ran a meta-analysis over all simulated sets. The power was calculated as the fraction of simulated meta-analysis p-values that was lower than the p-value threshold of 0.017.

Results

We excluded the study of Nagy et al. (Nagy et al. 2008) as this was not based on a case-control comparison. Additionally we removed the study of Yosifova et al. (Yosifova et al. 2009) as no effect size estimates were available here. Effect size estimates for the remaining studies as well as meta-analysis results are given in Table 3. The meta-analysis revealed associations for the allelic model, dominant and hethom model, all withstanding the threshold

of correction for multiple testing (p=0.011 allelic model, p=0.008 dominant model, p=0.009 hethom model). Forest plots and funnel plots for all three models are depicted in Figure 2. Inspection of funnel plots and Egger's test for asymmetry yielded no evidence for publication bias (p=0.206 allelic model, p=0.161 dominant model, p=0.286 hethom model).

In the meta-analysis, we had a power of 89% to detect associations between rs2230912 and mood-disorders withstanding correction for multiple testing.

Stratifying by disorder was possible for all studies except for Lavebratt et al. and Hejjas et al. where no disease-specific individual genotype distributions nor effect size estimates were given. In the meta-analysis of 4,182 MDD cases versus 5,926 controls (including the studies of Lucae et al., Green et al., Grigoroiu-Serbanescu et al. –German sample, Soronen et al., Vikii et al., Halmai et al. and the MARS study), all models were nominal significant (p=0.002 allelic, p=0.037 dominant, p=0.028 hethom model), however only the allelic model withstood correction for multiple testing.

For BD only, the total sample size was 3,813 cases versus 6,691 controls (Barden et al., Green et al., McQuillin et al., Grigoroiu-Serbanescu et al. , Soronen et al., Halmai et al., Backlund et al.). This resulted in no significant p-values in the meta-analysis (p=0.136, allelic, p=0.135, dominant, p=0.271 hethom model). Effect sizes for MDD were higher (OR of 1.12 for the allelic model in the meta-analysis) as compared to BP (OR of 1.05 for the same model). As most of the studies looked at a combined MDD/BP case sample in comparison to controls, separation of controls was not possible. Hence, most of the controls overlap between the stratified MDD and BD meta-analysis.

Discussion

In this meta-analysis we revisit the controversial association findings which have been reported for rs2230912 and mood disorders.

Although Feng et al. (Feng et al. 2014) reported lack of association in their meta-analysis in case-control samples, we found that rs2230912 was significantly associated with MDD/BD. Our study was well-powered, our result withstood correction for multiple testing and we did not find any evidence for publication bias.

This contradictory result could be explained by the fact that the set of studies included in our analysis differs from the studies included by Feng and colleagues. They excluded the results from Lucae et al. (Lucae et al. 2006) due to deviation from Hardy-Weinberg-Equilibrium (HWE). Different reasons could be responsible for this deviation. First, this could be due to genotyping error. However, as Lucae et al. mention in their publication, they re-genotyped rs2230912 using a different genotyping technique and could confirm the genotypic distribution. Second, if heterozygotes represented with the high-risk genotype and if controls were screened for mental disorders, a lack of heterozygotes in the control group can be expected. In that case, the control group would not be in the HWE. This is also in line with the study of Aprile-Garcia et al. (Aprile-Garcia et al. 2016) who showed that the heterozygous state of the SNP impairs receptor function with respect to calcium influx, channel currents and intracellular signaling and hence this state is probably the risk state. Furthermore, rs2230912 is just slightly out of HWE (p=0.03), and this p-value would not withstand correction for multiple testing over all included studies, we therefore decided to leave the results from Lucae et al. in our analysis. To clarify if our meta-analysis was mainly driven by the study of Lucae et al., we also ran an analysis after exclusion of the Lucae study. The allelic model was still nominally significant (p=0.031) while other models were not (p=0.089 dominant model, p=0.191 hethom model). The result for the allelic model did not survive multiple testing correction. However, one should take into account that this study was also one of the largest one with over 2,000 individuals. The fact that we detected only nominal significance could hence also be due to the reduced sample size.

No individual genotype counts nor effect size estimates were available for the study by Yosifova and colleagues (Yosifova et al. 2009) to us, therefore we had to exclude these findings from our analysis.

In comparison to Feng et al. we added a new study (Halmai et al. 2013) to the analysis as well as a new dataset for which results on rs2230912 have not been published before. Feng et al. excluded the study of Backlund et al. due to "duplicate report". However we checked with the authors of the studies published by Backlund et al. and Lavebratt et al. and they ensured that samples for the case-control analyses did not overlap. Therefore we kept both studies in the meta-analysis.

A possible critique of our study might be that we included studies on major depression as well as on bipolar disorder. We were interested whether rs2230912 was associated with mood disorders in general. Furthermore, there is a strong phenotypic overlap between both groups of patients. For those studies, where stratifying into MDD and BP was possible, we additionally ran separate meta-analyses. For the MDD sub-cohort the allelic model withstood correction for multiple testing, while no model was significantly associated for the BD sub-cohort. While we found comparable effect sizes for the combined MDD/BP sample as Feng et al., our findings with regard to stratification differ. While Feng et. al report an OR of 1.01 for MDD only and of 1.08 for BP only, we observed larger effect sizes for the MDD-cohorts (OR=1.14 for the allelic model) as compared to the BP cohort (OR=1.05 for the allelic model).

We focused on one single SNP in our meta-analysis. A broader approach involving more SNPs and ideally tagging all variation of the *P2RX7* locus will be necessary to determine to what extent *P2RX7* itself is involved in mood disorders.

Another important point needs to be addressed. While some cohorts (Lucae et al., Hejjas et al., McQuillin et al., Grigoroiu et al. – Romanian subsample, Lavebratt et al., Soronen et al., Viiki et al., Halmai et al., MARS study) used screened controls, all other studies included in

our meta-analysis included unscreened controls. With the prevalence of 16% for MDD and of 1% for BP in the general population, we cannot exclude that some of these controls were in fact suffering from mood disorders. If we restricted our meta-analysis to the cohorts with screened controls only, in general our effect size estimates increased and the meta-analysis p-values decreased. Future studies should therefore focus on screened controls, otherwise results might be biased.

Furthermore, we included studies which explicitly investigated if P2RX7, and rs2230912 specifically, was associated with mood disorders. Large genome-wide scale consortial studies of course also implicitly checked for association with this SNP. However, using the public available data from the Psychiatric Genomics Consortium (Major Depressive Disorder Working Group of the Psychiatric et al. 2013; Psychiatric 2011) whose MDD cohort also contains the sample of Lucae et al and the MARS study, rs2230912 was not significantly associated with neither MDD nor BP. The PGC is one of the largest studies to date that examined genome-wide associations with MDD or BP. Together with the great benefit of the very large sample-size, the cohorts are also very heterogenous and mostly used unscreened population controls and often non-clinical cases. Effects of rs2230912 might only present in more homogenous samples with regard to disease status, disease severity and screening.

Taking all our findings together and considering that a minor change in included studies led to different results in the meta-analysis, it seems evident that more studies are necessary to determine if rs2230912 is associated with mood disorders. Ideally these studies should be recruited as homogenously as possible. In future, the inclusion of larger studies may also allow to look more closely into whether our findings with regard to higher effect-sizes for MDD as compared to BP can be replicated and also to stratify by sex or different ethnicities. Furthermore, more focused studies on the SNP-effect in animal models could help to enlighten which genetic model exactly describes the association in the best way.

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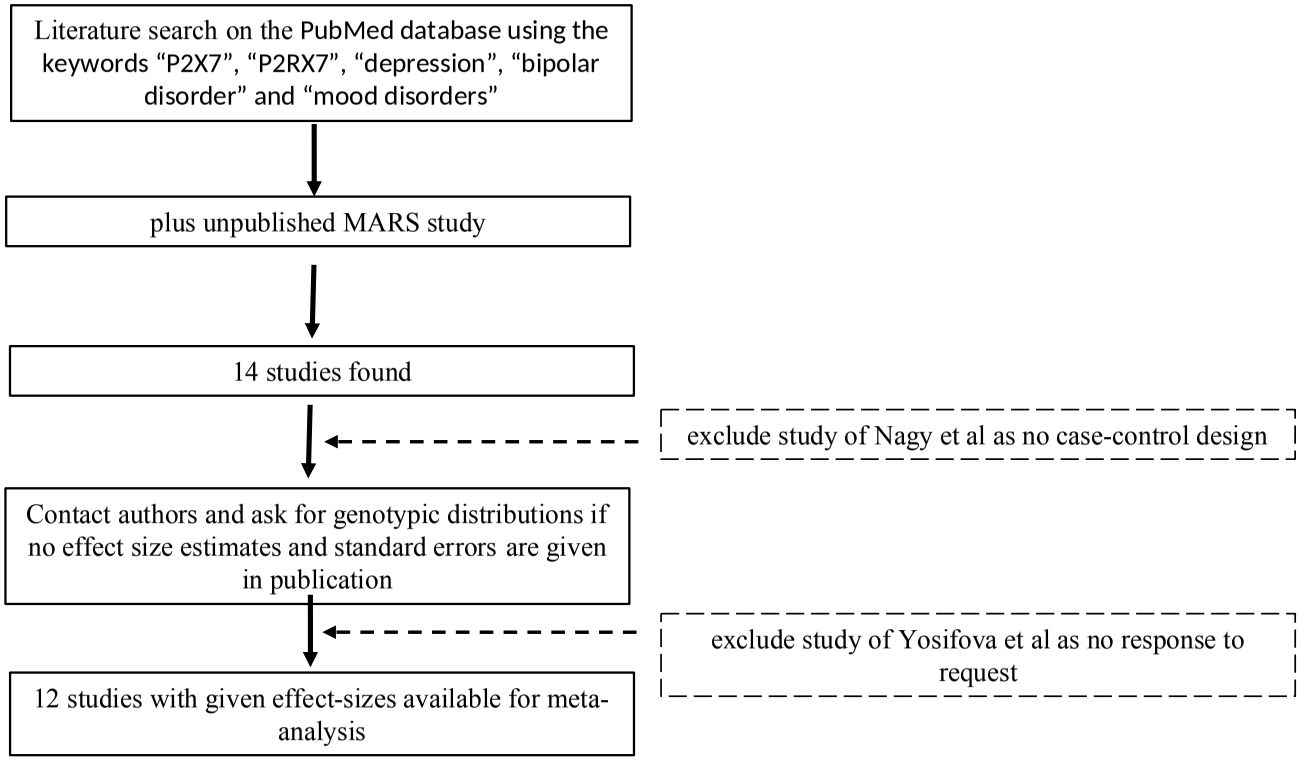
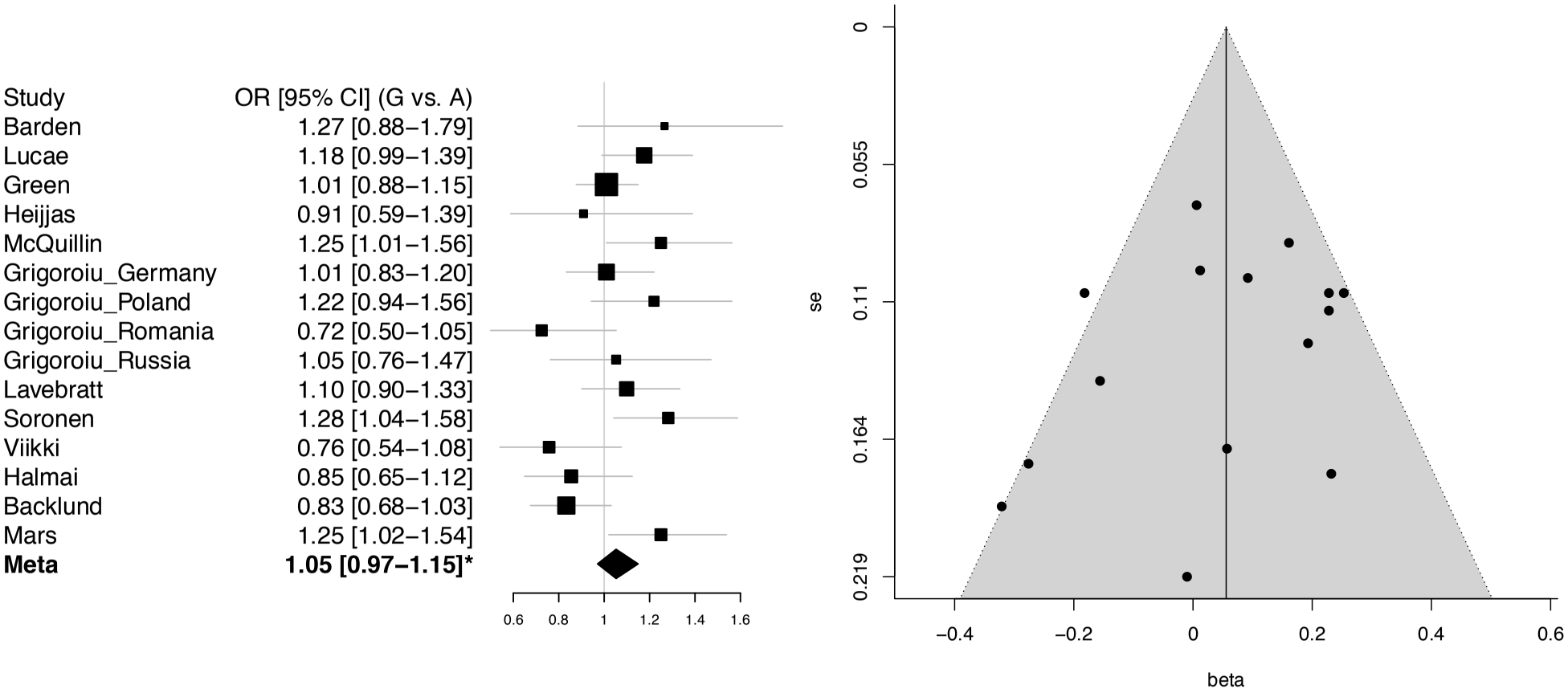


Figure 1: Flow diagram of meta-analysis

Figure 2: Forest plot and funnel plot of meta-analysis

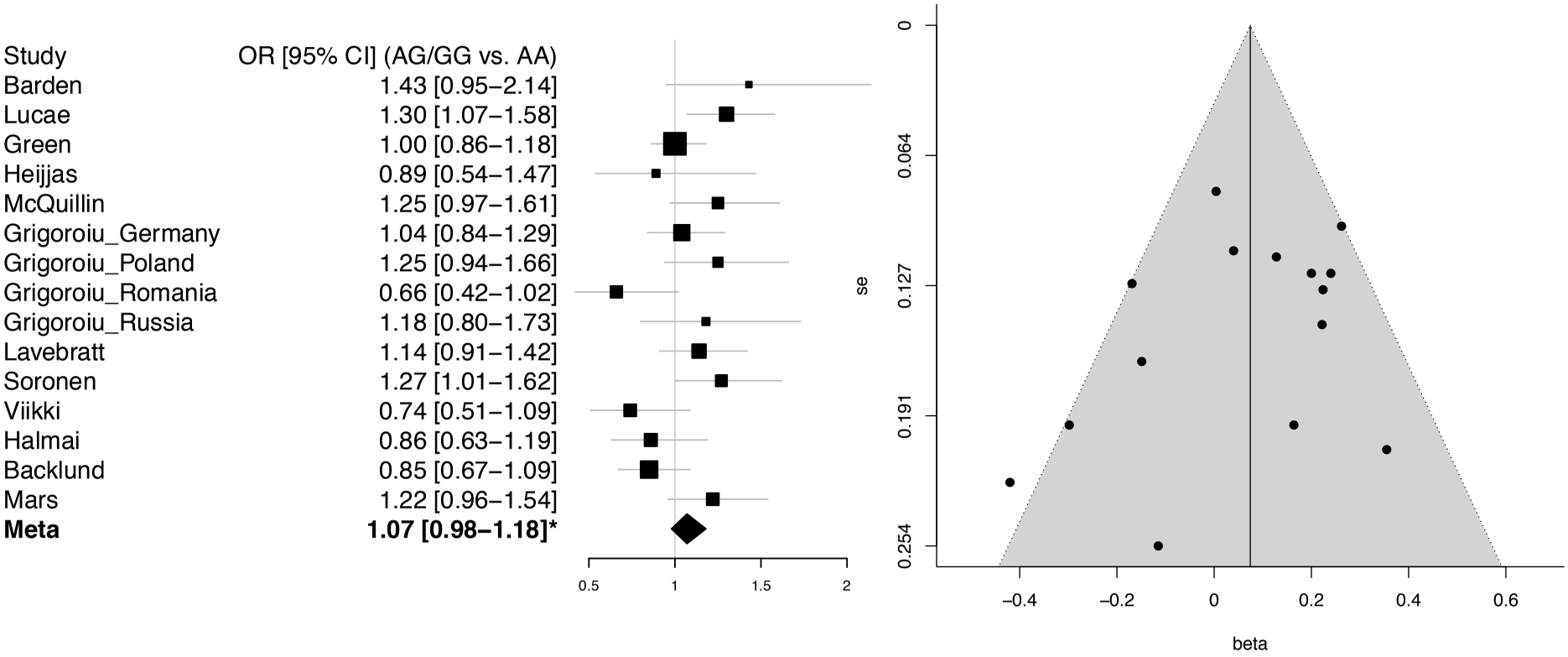
a) allelic model



*the Metasoft method of Han and Eskin (Han and Eskin 2011) gives the same estimate for effect size and confidence interval as the classical random-effects approach. Only the p-value differs as it is computed under a different null hypothesis. Therefore, meta-analysis p-values are significant although the confidence interval from the classical random-effects approach include an OR of 1.

Figure 2: Forest plot and funnel plot of meta-analysis

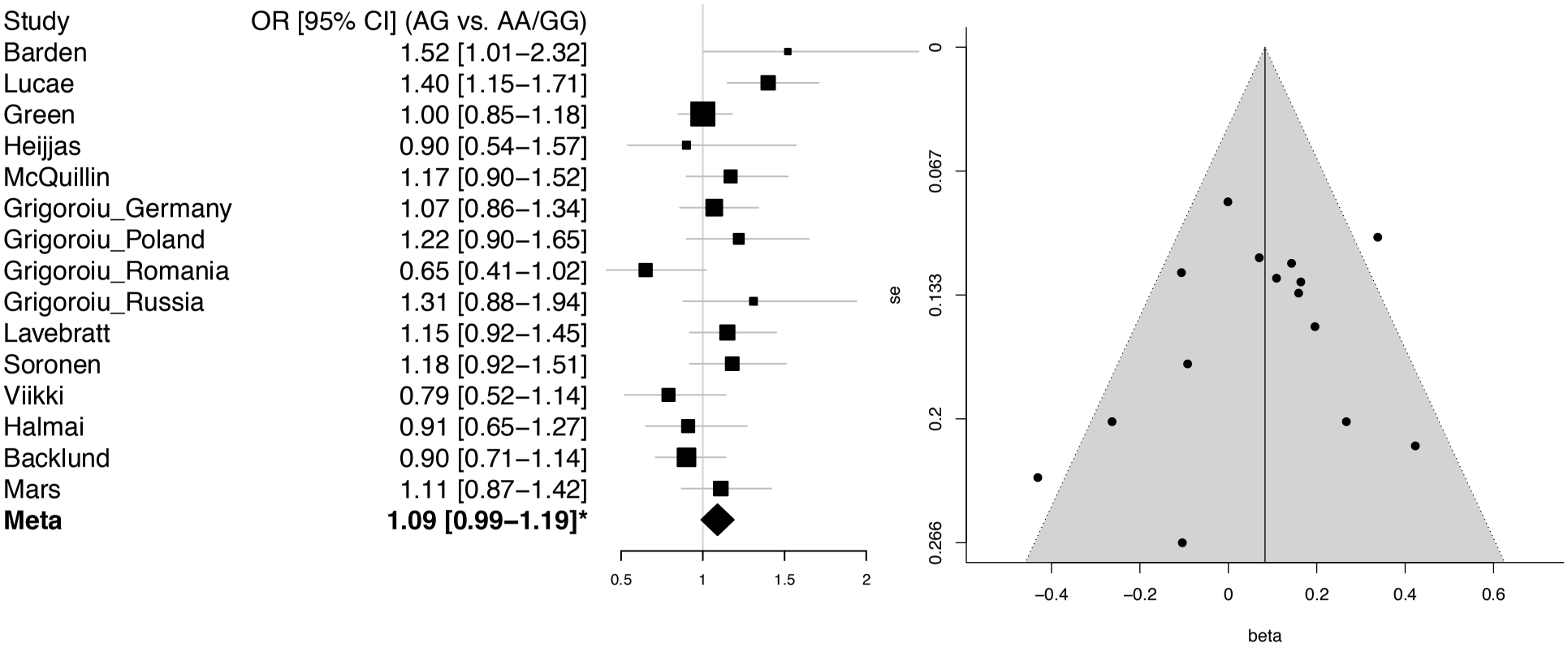
b) dominant model



*the Metasoft method of Han and Eskin (Han and Eskin 2011) gives the same estimate for effect size and confidence interval as the classical random-effects approach. Only the p-value differs as it is computed under a different null hypothesis. Therefore, meta-analysis p-values are significant although the confidence interval from the classical random-effects approach include an OR of 1

Figure 2: Forest plot and funnel plot of meta-analysis

b) hethom model



*the Metasoft method of Han and Eskin (Han and Eskin 2011) gives the same estimate for effect size and confidence interval as the classical random-effects approach. Only the p-value differs as it is computed under a different null hypothesis. Therefore, meta-analysis p-values are significant although the confidence interval from the classical random-effects approach include an OR of 1

Table 1: demographics of MARS study

	unipolar cases	controls	p-value
n	832	540	
n male	401 (48.2%)	244 (45.2 %)	0.31 [a]
age mean (sd)	48.31 (14.09)	47.39 (13.50)	0.23 [b]

[a] Based on X^2-Square test for independence
[b] Based on t-test for difference of means

Table 2: studies included in the meta-analysis

publication	# cases	type cases	# controls	reported association with rs2230912
Barden et al., 2006	213	BD	214	yes
Lucae et al., 2006	1,000	MDD	1,029	yes
Green et al., 2009	1,723	BD/MDD	1,204	no
Hejjas et al., 2009	171	BD/MDD	178	no
McQuillin et al., 2009	613	BD	560	yes
Grigoroiu-Serbanescu et al., 2009	2,091	BD/MDD	2,008	no
Yosifova et al., 2009[a]	172	BD	556	no
Lavebratt et al., 2010	457	BD/MDD	2,286	no
Soronen et al, 2011	450	BD/MDD	1,322	yes
Vikii et al, 2011	218	MDD	395	no
Halmai et al., 2013	315	BD/MDD	373	no
Backlund et al., 2012 [b]	569	BD	1,044	not given
MARS study	832	MDD	540	not published

[a] No individual genotypes were available for this study therefore it was not included in further analyses

[b] This publication studied rapid cycling and not BD per se. However, genotypic distributions for cases and controls were given, therefore we included the study in further analyses.

Table 3: meta-analysis results

Study	allelic model G vs. A			dominant model AG/GG vs. AA			hethom model AG vs. AA/GG		
	beta	se	p-value	beta	se	p-value	beta	se	p-value
Barden et al.	0.232	0.178	0.194	0.355	0.207	0.086	0.423	0.214	*0.048*
Lucae et al.	0.161	0.086	0.061	0.262	0.098	**0.007**	0.338	0.102	**0.001**
Green et al.	0.006	0.071	0.936	0.004	0.081	0.966	-0.001	0.083	0.995
Hejjas et al.	-0.010	0.219	0.648	-0.115	0.254	0.652	-0.104	0.266	0.696
McQuillin et al.	0.228	0.113	*0.043*	0.224	0.129	0.081	0.159	0.132	0.229
Grigoroiu-Serbanescu et al.: German sample	0.012	0.097	0.906	0.040	0.110	0.714	0.070	0.113	0.538
Grigoroiu-Serbanescu et al.: Polish sample	0.193	0.126	0.126	0.222	0.146	0.130	0.196	0.150	0.193
Grigoroiu-Serbanescu et al.: Romanian sample	-0.321	0.191	0.093	-0.420	0.223	0.060	-0.431	0.231	0.062
Grigoroiu-Serbanescu et al.: Russian sample	0.057	0.168	0.736	0.164	0.195	0.400	0.267	0.201	0.184
Lavebratt et al.	0.092	0.100	0.354	0.128	0.113	0.260	0.143	0.116	0.216
Soronen et al.	0.253	0.106	*0.017*	0.240	0.121	*0.048*	0.164	0.126	0.192
Vikii et al.	-0.276	0.174	0.112	-0.298	0.195	0.125	-0.263	0.201	0.189
Halmai et al.	-0.156	0.141	0.267	-0.149	0.164	0.364	-0.092	0.170	0.589
Backlund et al.	-0.182	0.106	0.087	-0.169	0.126	0.164	-0.106	0.121	0.381
MARS study	0.228	0.106	*0.032*	0.200	0.121	0.097	0.109	0.124	0.380
Meta-analysis	0.056	0.044	**0.011**	0.074	0.049	**0.008**	0.082	0.048	**0.009**

Beta is given as log(odds ratio) with the respective se (standard error) of the log(odds ratio)
p-values < 0.05 are depicted in italic
p-values < 0.017, hence surviving multiple-testing over all models, are depicted in bold.