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Translating GWAS findings into therapies for depression and anxiety disorders: Drug repositioning using gene-set analyses and testing for enrichment of psychiatric drug classes

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Abstract

Depression and anxiety disorders are the first and sixth leading cause of disability worldwide according to latest reports from the World Health Organization. Despite their high prevalence and the significant disability resulted, there are limited advances in new drug development. On the other hand, the advent of genome-wide association studies (GWAS) has greatly improved our understanding of the genetic basis underlying psychiatric disorders.

In this work we employed gene-set analyses of GWAS summary statistics for drug repositioning. We explored five related GWAS datasets, including two on major depressive disorder (MDD-PGC and MDD-CONVERGE, with the latter focusing on severe depression cases), one on anxiety disorders, and two on depressive symptoms and neuroticism in the population. We extracted gene-sets associated with each drug from DSigDB and examined their association with each GWAS phenotype. We also performed repositioning analyses on meta-analyzed GWAS data, integrating evidence from all related phenotypes.

Importantly, we showed that the repositioning hits are generally enriched for known psychiatric medications or those considered in clinical trials, except for MDD-PGC. Enrichment was seen for antidepressants and anxiolytics but also for antipsychotics. We also revealed new candidates for repositioning, some of which were supported by experimental or clinical studies. For example, the top repositioning hit using meta-analyzed p-values was fendiline, which was shown to produce antidepressant-like effects in mouse models by inhibition of acid sphingomyelinase and reducing ceramide levels. Taken together, our findings suggest that human genomic data such as GWAS might be useful in guiding drug discoveries for depression and anxiety disorders.

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Introduction

Depression and anxiety disorders are among the most common psychiatric disorders. According to the latest report by the World Health organization, depression affects more than 300 million people worldwide and is the leading cause of disability[1]. Anxiety disorders affects more than 260 million people and is the sixth leading cause of disability[1]. The two disorders are highly comorbid and might share common pathophysiologies[2,3]. Nevertheless, pharmacological treatment for major depressive disorder (MDD) or anxiety disorders (AD) has not seen much advance in the last two decades or so, with a lack of therapies having novel mechanisms of action. In addition, only about one third of MDD patients achieve complete remission after a single antidepressant trial[4] and around 10 to 30% of patients are treatment-resistant[5].

On the other hand, with the advent of high-throughput technologies such as genome-wide association studies (GWAS) in the last decade, we have gained a much better understanding of the genetic bases of many complex diseases. It is hoped that human genomics data will accelerate drug development for psychiatric disorders, especially due to the difficulties for animal models to fully mimic human psychiatric conditions[6].

Conventional drug development is a very lengthy and costly process. An alternative is to explore existing drugs for new disease indications, or drug repositioning. A repurposed drug can be brought into clinical use in a much shorter time-frame than a brand-new medication[7]. In this study we will take advantage of large-scale GWAS summary data for drug repositioning.

The majority of GWAS studies focus on the identification of new susceptibility loci and relatively few have explored the potential of using the ever-growing data to guide drug discoveries. In an earlier study, Sanseau et al.[8] identified the most significant GWAS hits from a range of diseases and compared them against known drug targets to find "mismatches" (i.e. drug indication different from the studied disorder) as candidates for repurposing. While it is intuitive to focus on the most significant SNPs, for many complex traits the genetic architecture may be highly polygenic and variants of weaker effects may be "hidden". Moreover, given the complex and multifactorial etiologies of many complex diseases, the development of multi-target drugs with wide-ranging biological activities (known as "polypharmacology") is gaining increased attention (please refer to e.g. Anighoro et al.[9] for a review). It is argued that multi-target drugs may have improved efficacy over highly selective pharmacological agents, as they tackle multiple pathogenic pathways in the system.

In this study we employed gene-set analysis (GSA) for drug repositioning. Here the gene-sets are defined as genes related to a drug, for example known drug targets, genes

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differentially expressed after drug treatment or genes with active bioassay results. We considered a large panel of drugs and investigated whether the gene-set associated with each drug is enriched among GWAS results. The top results can then serve as candidates for repurposing. We also tested whether the top repositioned candidates are enriched for known psychiatric medications.

As will be discussed later, the current study is also complementary to our recent drug repositioning attempt by a new general methodology in which expression profiles of drugs are compared against those of diseases[10]. This study employed GSA instead and gene-based statistics were directly derived from GWAS results, attacking the problem from a different angle.

Gene-set analysis is an established approach to gain biological insight into expression microarrays, GWAS or other high-throughput "omics" studies[11,12]. De Jong et al. made use of GSA to identify repurposing opportunities for schizophrenia[13]. In another very recent study, Gasper et al.[14] performed further analyses of GSA results, and reported that GWAS signals of schizophrenia are enriched for neuropsychiatric medications as sample size increases. Another related study on schizophrenia was conducted by Ruderfer et al., who collected genome-wide significant GWAS variants and exome sequencing results and compared the identified genes against drug targets. Significant enrichment was noted for antipsychotics.

In this work we take a different focus on depression and anxiety disorders, which are highly prevalent and disabling disorders. We consider not only one but multiple GWAS summary datasets with additional analyses on psychiatric drug class enrichment.

Methods

Genome-wide association studies data

We considered five GWAS datasets that are associated with depression and anxiety. Two are studies of major depressive disorder (MDD), namely MDD-PGC[15] and MDD-CONVERGE[16]. However, the two studies are different in a number of ways. The MDD-PGC sample is composed of Caucasians of both sexes, while MDD-CONVERGE is a cohort of Chinese women. The MDD-CONVERGE sample mainly consists of hospital-ascertained cases affected by severe depression, of whom ~85% had melancholic symptoms[16]. The MDD-PGC sample on the other hand is more heterogeneous and not specifically enriched for any subtypes of depression[15].

Another two GWAS studies were meta-analyses on depressive symptoms and neuroticism conducted by the Social Science Genetics Association Consortium (SSGAC)[17]. The

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meta-analysis on depressive symptoms (SSGAC-DS) included the MDD-PGC sample, but it also contained general population samples. We also included another study on neuroticism (SSGAC-NEU), as this personality trait is known to be closely associated with depression and anxiety disorders[18]. In addition, antidepressants may affect personality traits, including a reduction in neuroticism, independent of their effects on depressive symptoms[19].

The fifth dataset is a GWAS meta-analysis of anxiety disorders, including generalized anxiety disorder, panic disorder, social phobia, agoraphobia, and specific phobias[20]. We extracted the GWAS results of case-control analyses.

GWAS summary results were downloaded from https://www.med.unc.edu/pgc/results-and-downloads and https://www.thessgac.org/data.

Extracting gene-sets associated with each drug

We made use of the DSigDB database[21] to extract gene-sets related to each drug. DSigDB holds gene-sets for a total of 17839 unique compounds. The gene-sets are compiled according to multiple sources: (1) bioassay results from PubChem[22] and ChEMBL[23]; (2) kinase profiling assay from the literature and two kinase databases (Medical Research Council Kinase Inhibitor database and Harvard Medical School Library of Integrated Network-based Cellular Signatures database); (3) differentially expressed genes after drug treatment (with >2 fold-change compared to controls), as derived from the Connectivity Map[24]; and (4) manually curated and text mined drug targets from the Therapeutics Targets Database (Qin et al., 2014) and the Comparative Toxicogenomics Database[25]. We downloaded the entire database from http://tanlab.ucdenver.edu/DSigDB.

It should be noted that although the focus is on drug "repositioning", the analytic framework is general and can apply to any drugs with some known associated genes. Indeed DSigDB contains a substantial number of drugs which do not have an approved indication yet; they are still included in our analyses.

Gene-set analysis (GSA) approach

We first converted the SNP-based test results to gene-based test results. We employed fastBAT[26] (included in the software package GCTA) for gene-based analyses. FastBAT computes the sum of chi-square statistics over all SNPs within a gene and uses an analytic approach to compute the *p*-value. Gene size and linkage disequilibrium patterns are taken into account when computing the *p*-values. The same statistical approach for gene-based tests is also used by two other popular programs, VEGAS[27] and PLINK[28], although they computed *p*-values by simulations or permutations. FastBAT has been shown to be equivalent to VEGAS

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and PLINK at higher *p*-values (>1E-6) and more accurate than them for smaller *p*[26]. We ran fastBAT with default settings and used the 1000 Genome genotype data as the reference panel.

We then performed a standard GSA by comparing gene-based test statistics within and outside the gene-set. We adopted the same approach as implemented in MAGMA[29], which is also reviewed in [12]. Briefly, gene-based *p*-values are first converted to z-statistics by $z = \Phi^{-1}(p)$, where Φ^{-1} is the probit function (more negative z-values represent stronger statistical associations). We then employed a single-sided two-sample *t*-test to see if the mean *z*-statistics of genes within the gene-set is lower than that outside the gene-set. To avoid results driven by only a few genes, we only considered drugs with at least 5 genes in their gene-sets. A total of 5232 drugs were included for final analyses.

Combining p-values across datasets

Besides analyzing each GWAS dataset in turn for repositioning opportunities, we also considered the aggregate contribution of all datasets, as depression, anxiety and neuroticism are closely connected to each other. We performed meta-analysis of *p*-values based on two methods, the Simes' method[30] and the Brown's approach[31]. The Simes' method is valid under positive regression dependencies[32]. Brown's method is similar to Fisher's method but accounts for dependencies in *p*-values. The MDD-CONVERGE sample includes only Chinese subjects and does not overlap with other datasets, but we accounted for overlapping samples in the remaining GWAS studies. We did not include MDD-PGC when combining *p*-values as this sample is already included in the GWAS results of depressive symptoms.

Testing for enrichment of psychiatric drug classes

We considered three sources for defining psychiatric drug-sets in our analyses. The first set comes from the Anatomical Therapeutic Classification (ATC) drugs downloaded from KEGG. We extracted three groups of drugs: (1) all psychiatric drugs (coded "N05" or "N06"); (2) antipsychotics (coded "N05A"); (3) antidepressants and anxiolytics (coded "N05B" or "N06A"). We did not specifically include drugs for dementia or psychostimulants as they are relatively small in number. The second source is from MEDication Indication resource (MEDI)[33] derived from four public medication resources, namely RxNorm, Side Effect Resource 2 (SIDER2), Wikipedia and MedlinePlus. A random subset of the extracted indications was checked by physicians. The MEDI high-precision subset (MEDI-HPS), with an estimated precision of 92%, was used in our analyses[33]. Since only known drug indications are included in ATC or MEDI-HPS, we also included an expanded set of drugs that are considered for clinical trials (as listed on https://clinicaltrials.gov). These drugs are usually promising candidates supported by preclinical or clinical studies. A precompiled list of these drugs (created in May 2016) was obtained from https://doi.org/10.15363/thinklab.d212. For ATC and

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MEDI-HPS, due to the relatively small number of drugs in each class, we combine schizophrenia with bipolar disorder (BD), as well depression with anxiety.

We performed enrichment tests of repositioning hits for known drug classes, in a manner similar to the GSA described above. P-values are first converted to *z*-statistics, and the mean z-score within each drug class is compared against the theoretical null of zero (self-contained test) and against other drugs outside the designated drug class (competitive test) with one-sided tests.

It is reasonable to believe that the current antidepressants or anxiolytics are not the only drugs that have therapeutic effects; in other words, a certain proportion of drugs in the "competing set" might also have therapeutic potential against depression or anxiety. Therefore, results of the competitive tests should be interpreted with this potential limitation in mind. In this paper we presented the drug-set enrichment results of both self-contained and competitive tests.

Literature search

For the top 30 repositioning hits based on meta-analyzed GWAS results (Brown's or Simes' method), we searched PubMed and Google scholar using the following terms: Drug_name AND (depression OR depressive OR antidepressant OR anxiety OR panic OR phobia OR anxiolytic). We also looked up the references therein if relevant.

Correction for multiple testing

We employed the false discovery rate (FDR) approach (which controls the expected proportion of false positives among those declared to be significant) to account for multiple testing[34]. FDR-adjusted *p*-values (or *q*-values) were computed by the R function p.adjust with the Benjamini-Hochberg (BH) procedure[34]. The primary *q*-value threshold was set at 0.05, while $q < 0.1$ was regarded as suggestive association.

RESULTS

Enrichment of psychiatric drug classes among the drugs repositioned from gene-set analyses

Table 1-3 and Supplementary Tables 1-3 show the enrichment *p*-values and *q*-values for major psychiatric drug classes amongst the drugs repositioned from GSA. We observed that the drugs repositioned from most GWAS of anxiety and depressive traits are enriched for known psychiatric medications.

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First we consider the three datasets (MDD-CONVERGE, MDD-PGC, SSGAC-DS) which focus on depression traits (Table 1 and Supplementary Table 1). On the whole the MDD-CONVERGE sample showed the strongest enrichment with the greatest number of significant results. Significant enrichment was seen for antipsychotics and antidepressants or anxiolytics within ATC and MEDI-HPS categories. Interestingly, when considering drugs included for clinical trials, the enrichment was more specific for anxiety and depression, while no significant results were found for schizophrenia.

In contrast, we did *not* observe any significant enrichment for drugs repositioned from the MDD-PGC sample. The SSGAC-DS study included MDD-PGC data but the latter only comprised ~10% of the total sample size. For SSGAC-DS, we observed enrichment of drugs for schizophrenia and BD, and suggestive associations with anxiety and depression for medications listed in clinicalTrial.gov.

As for neuroticism and anxiety disorders, there was evidence of enrichment in most drug classes under study. Interestingly, for neuroticism, the strongest enrichment was for antipsychotics (lowest q = 2.28E-09) instead of antidepressants. Table 2 and Supplementary Table 2 show the enrichment p-values and q-values respectively.

For analyses involving meta-analyzed GWAS data across all datasets (Table 3 and Supplementary Table 3), enrichment was observed for all psychiatric drug classes, with generally stronger or at least comparable statistical associations when compared to enrichment tests of individual GWAS. The results of Brown's and Simes' tests were largely consistent with each other.

Top repositioning hits

We found a few interesting repositioning hits that were supported by previous studies (Table 4; full results of drug repositioning are given in Supplementary Table 4). Here we focus our discussion on the meta-analyzed results integrating all associated GWAS datasets (using either Brown's or Simes' method) and drugs among the top 30 hits. The top repositioning hit identified in meta-analysis was fendiline (Brown's p = 1.06E-11, q = 5.55E-8), a non-selective calcium channel blocker. Fendiline was shown to exert antidepressant-like effects in a mouse model by inhibition of acid sphingomyelinase (ASM) activity and reduction of ceramide concentrations in the hippocampus[35]. A drop in ceramide concentrations might lead to increased neurogenesis and improved neuronal maturation and survival[35].

Another drug on the top list was alsterpaullone, a glycogen synthase kinase-3β (GSK-3β) inhibitor[36]. GSK-3β is involved in multiple psychiatric disorders, including depression[37]. Diniz

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et al. reported higher platelet GSK-3β activities in elderly depressive patients[38]. Increased activation of GSK-3β was also associated with depression-like behavior in mouse models, which could be alleviated by GSK-3β inhibitors[39]. In addition, inhibition of GSK3 has been postulated as a major mechanism of action by the mood stabilizer lithium[40].

Another drug sanguinarine, which is a selective mitogen-activated protein kinase phosphatase-1 (Mkp-1) inhibitor, was shown to produce antidepressant-like effect in rats[41]. Piperlongumine, a constituent of the fruit of *Piper longum*, was shown to confer resistance against stress in a mouse model[42].

Interestingly, among the top repositioning hits from the meta-analysis results, a number of them are calcium channel blockers (CCB). These include fendiline, perhexiline, prenylamine and felodipine (prenylamine was withdrawn from the market due to risk of QT prolongation and torsades de pointes[43]). Although with the exception of fendiline, no direct experimental or clinical studies have shown antidepressant or anxiolytic properties of the above drugs, CCB as a whole have been proposed as treatment for various psychiatric disorders. CCB has been mostly studied for the treatment of mania, recently reviewed in Cipriani et al.[44]. However the number of quality double-blind randomized controlled trials (RCT) was small, and there is yet insufficient evidence to suggest the use of CCB in treating manic symptoms. As for depression, a recent pilot (patient-only) study of isradipine on bipolar depression showed positive results[45]. Another CCB, nicardipine, was reported to enhance the antidepressant action of electroconvulsive therapy[46]. Two RCTs also showed nimodipine might be useful as an augmenting agent for vascular depression patients[47,48]. A limited number of studies have investigated the use of CCB in anxiety disorders, with some successes but negative results have also been reported[49]. Notwithstanding the mixed evidence, CCB are probably still worthy of further investigation for depression and anxiety disorders, given the biological relevance of calcium signaling and some support from clinical studies.

DISCUSSION

In this study we leveraged large-scale GWAS summary data and analyzed gene-sets associated with drugs to uncover repositioning opportunities for depression and anxiety disorders. It is encouraging that we observed significant enrichment for known psychiatric medications or drugs considered in clinical trials. To our knowledge, this is the first study to demonstrate such enrichment. It also provides support for the validity of GSA in drug repurposing. In addition, we reveal a few interesting candidates for repurposing that are supported by prior studies. Taken together, our findings support the usefulness of GWAS data in guiding drug discoveries. Although only few susceptibility variants of genome-wide significance have been found for depression and anxiety disorders, our findings suggest that leveraging variants with weaker

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associations, for example by GSA, might still contribute valuable information to the discovery of novel therapies.

It is noteworthy that while we have included three datasets (SSGAC-DS, MDD-PGC, MDD-CONVERGE) related to depression, the enrichment results are quite different. Both MDD-PGC and MDD-CONVERGE are case-control GWAS studies on MDD; however the PGC sample showed no significant enrichment for known psychiatric medications. The discrepancy might be due to the differences between the two samples. As described above, the two samples differ by gender, ethnicity and the severity of depression. In addition, due to the lower awareness and possibly stronger resistance to seeking medication attention for depression in China, the disease severity in the CONVERGE cohort may be even higher than expected. It is widely accepted that MDD is a heterogeneous disorder, with a variety of clinical presentations and possibly divergent pathophysiologies[50]. By recruiting a more homogeneous group of patients, the CONVERGE study might have better power in detecting susceptibility genes despite a lower sample size. Indeed, MDD-CONVERGE revealed two genome-wide significant loci while none was found in the MDD-PGC study. It is also worth mentioning that previous meta-analyses showed that the response to antidepressant depends on the baseline severity of depression[51,52]. They reported that effects of antidepressants were largest for the most severely depressed group, but small or even non-existent for mild to moderate depression. Our findings, although based on a different study paradigm, are broadly in line with this clinical observation.

The SSGAC-DS study is about 10 times the sample size of MDD-PGC. It contained two case-control samples but also included a study of depressive symptoms in the general population (UK BioBank study). Although SSGAC-DS is not selective for depression phenotypes, the significant enrichment results suggest that expanding the sample size may be one way to overcome the genetic and phenotypic heterogeneities for drug repositioning.

Also, although the two SSGAC studies involve symptom measures in the general population instead of clinically diagnosed depression or anxiety disorders, we still observe enrichment for psychiatric drug classes. Drug repositioning may hence benefit from genetic studies of less restrictive symptom traits in the population, in addition to clinically diagnosed samples. In addition, our results suggest that combined analyses of multiple associated traits may further improve the power to detect new repositioning opportunities.

It is noteworthy that the repositioning hits are not only enriched for antidepressants or anxiolytics but also antipsychotics. A meta-analysis by Spielmans et al. revealed that atypical antipsychotics are effective as adjunctive treatment for treatment-resistant depression[53]. Zhou

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et al. also reached a similar conclusion in a recent network meta-analysis[54]. Atypical antipsychotics may also be useful for anxiety disorders and symptoms[55-57], although further studies are still required and that the benefits need to be balanced against the side-effects. Furthermore, a shared genetic basis between schizophrenia and depression is well-established[58], and a recent study also found significant genetic correlation between neuroticism and schizophrenia[59].

In this study we employed the GSA approach to drug repositioning. The current study is complementary to our recent repositioning attempt by a novel methodology in which the drug-induced transcriptome are compared against GWAS-imputed expression profiles[10]. Each of these two methods has their own advantages and disadvantages. The methodology of finding reversed expression patterns has a unique advantage of accounting for the directions of associations. It also takes into account the functional impact of variants on expression and is intuitive from a biological point of view. While differentially expressed genes can be included in gene-sets, the actual (quantitative) expression changes are not considered which results in a loss of information. GSA also does not delineate the directions of effects. Nevertheless, GSA can make use of knowledge concerning known drug targets and other information on drug-related genes, for which more databases are available. Also the transcriptome comparison approach involves "imputing" expression levels; since the major reference transcriptome dataset (GTEx) is mainly composed of Caucasians (84.6%) with greater proportion of males (65.6%) (https://www.gtexportal.org/home/tissueSummaryPage, accessed 29th Apr 2017), the quality of imputation for other ethnicities and females may be less reliable, for example when applied to the MDD-CONVERGE dataset.

Just as medications acting on different pathways might have synergistic therapeutic effects, we believe that it is beneficial to have different approaches for computational drug repositioning to complement each other. Of course, computational methods leveraging human genomic data are not the only means to drug discoveries. We believe that a combination of a variety of approaches, including experimental and computational ones, is required to speed up drug repurposing and discoveries.

Our enrichment analyses support the application of GSA in drug repositioning in depression and anxiety. However, we stress that our repositioning results should be validated in further pre-clinical and clinical studies before translation to practice. GSA analyses do not provide information on the direction of effects, as discussed previously. Measures of statistical significance also do not provide direct or definitive evidence for the actual therapeutic effects of the repositioned drugs.

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In summary, we have performed a drug repositioning analyses on depression and anxiety disorders, using a gene-set analysis approach considering five related GWAS studies. We showed that the repositioned drugs are in general enriched for known psychiatric medications or those considered in clinical trials. The results lend further support to the usefulness of human genomic data in guiding drug development in psychiatry, and we hope that the rapid advances in psychiatric genomics research will translate into benefits for patients in the foreseeable future.

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Table 1 Enrichment *p*-values of repositioning hits derived from GWAS of major depressive disorder (MDD) and depressive symptoms

Disorder	MDD-CON Self	MDD-CON Compet	MDD-PGC Self	MDD-PGC Compet	DepSym Self	DepSym Compet
ATC classification						
Antipsychotics	**2.50E-04**	**1.13E-02**	1.00E+00	9.98E-01	**1.35E-03**	*4.48E-02*
Antidepressants or anxiolytics	**2.10E-06**	**7.64E-04**	1.00E+00	9.82E-01	**1.90E-02**	3.44E-01
All ATC psychiatric drugs	**3.54E-08**	**1.83E-03**	1.00E+00	9.98E-01	**5.17E-07**	**9.62E-03**
MEDI-HPS						
Schizophrenia and Bipolar	**3.06E-07**	**2.04E-04**	1.00E+00	1.00E+00	**2.90E-02**	4.68E-01
Anxiety and Depression	**2.98E-07**	**6.59E-04**	1.00E+00	9.99E-01	**2.08E-03**	2.02E-01
All psychiatric drugs	**1.77E-10**	**4.40E-05**	1.00E+00	1.00E+00	**1.35E-03**	3.15E-01
ClinicalTrial.gov						
Anxiety disorders	**3.20E-07**	**2.18E-03**	1.00E+00	9.92E-01	**4.97E-04**	1.16E-01
Depression	**9.45E-07**	**2.27E-02**	1.00E+00	9.99E-01	**5.76E-05**	8.67E-02
Bipolar disorder	**1.64E-03**	1.69E-01	1.00E+00	9.97E-01	**1.55E-05**	**9.33E-03**
Schizophrenia	**5.42E-05**	9.66E-02	1.00E+00	9.96E-01	**4.39E-05**	9.42E-02
Anxiety + Depression	**3.14E-09**	**1.69E-03**	1.00E+00	1.00E+00	**1.23E-05**	*6.07E-02*
Schizophrenia + Bipolar	**2.62E-05**	9.12E-02	1.00E+00	9.96E-01	**4.85E-07**	**8.78E-03**
All psychiatric drugs	**6.89E-11**	**1.43E-03**	1.00E+00	1.00E+00	**5.09E-09**	**1.49E-02**

Self: self-contained test; Compet, competitive test. Test results with *q*-value < 0.05 are in bold. Results with *q*-value between 0.05 and 0.1 are in italics. Full tables of *q*-values are presented in Supplementary Table 1.

MDD-CONVERGE, MDD with GWAS data from the CONVERGE Consortium; MDD-PGC, MDD with GWAS data from the Psychiatric Genomics Consortium; DepSym, GWAS of depressive symptoms from the Social Science Genetics Association Consortium (SSGAC).

Table 2 Enrichment p-values of repositioning hits derived from GWAS of anxiety disorders and neuroticism

Disorder	AnxietyCC Self	AnxietyCC Compet	Neurotic Self	Neurotic Compet
ATC classification				
Antipsychotics	**1.71E-03**	**4.47E-04**	**1.21E-10**	**1.52E-10**
Antidepressants or anxiolytics	**1.35E-02**	**5.23E-03**	**1.09E-03**	**1.45E-03**
All ATC psychiatric drugs	**6.67E-03**	**7.93E-04**	**4.88E-12**	**7.99E-12**
MEDI-HPS				
Schizophrenia and Bipolar	**7.51E-03**	**2.30E-03**	**1.29E-06**	**1.98E-06**
Anxiety and Depression	**8.24E-03**	**2.46E-03**	**5.98E-05**	**9.01E-05**
All psychiatric drugs	**4.18E-04**	**3.82E-05**	**1.02E-07**	**1.77E-07**
ClinicalTrial.gov				
Anxiety disorders	**2.84E-02**	**7.94E-03**	**2.70E-03**	**3.82E-03**
Depression	**1.17E-02**	**1.55E-03**	8.62E-02	1.14E-01
Bipolar disorder	**1.56E-02**	**3.53E-03**	**5.93E-03**	**7.94E-03**
Schizophrenia	**3.49E-03**	**3.43E-04**	**9.70E-04**	**1.47E-03**
Anxiety + Depression	**1.78E-02**	**2.33E-03**	*4.09E-02*	*5.70E-02*
Schizophrenia + Bipolar	**3.12E-03**	**2.69E-04**	**5.32E-04**	**7.84E-04**
All psychiatric drugs	**8.26E-03**	**3.61E-04**	**2.16E-03**	**3.34E-03**

Self: self-contained test; Compet, competitive test. Test results with q-value < 0.05 are in bold. Results with q-value between 0.05 and 0.1 are in italics. Full tables of q-values are presented in Supplementary Table 2.

Anxiety CC, GWAS of anxiety disorders case-control sample; neurotic, GWAS of neuroticism in general population.

Table 3 Enrichment p-values of repositioning hits derived from meta-analysis of GWAS *p*-values from MDD-CONVERGE, MDD-PGC, SSGAC-DS and SSGAC-NEU

Disorder	Brown Self	Brown Compet	Simes Self	Simes Compet
ATC classification				
Antipsychotics	**2.31E-09**	**8.77E-07**	**4.64E-07**	**9.11E-06**
Antidepressants or anxiolytics	**8.30E-10**	**3.19E-06**	**2.01E-07**	**9.57E-06**
All ATC psychiatric drugs	**2.51E-18**	**3.16E-10**	**8.38E-12**	**3.03E-08**
MEDI-HPS				
Schizophrenia and Bipolar	**5.19E-08**	**5.70E-05**	**7.14E-06**	**1.79E-04**
Anxiety and Depression	**1.63E-10**	**3.18E-06**	**1.35E-06**	**8.95E-05**
All psychiatric drugs	**2.36E-13**	**2.85E-07**	**1.83E-08**	**6.63E-06**
ClinicalTrial.gov				
Anxiety disorders	**3.47E-08**	**4.63E-04**	**1.98E-04**	**7.49E-03**
Depression	**4.33E-10**	**3.82E-04**	**1.04E-06**	**6.14E-04**
Bipolar disorder	**5.53E-09**	**7.43E-05**	**8.07E-07**	**7.47E-05**
Schizophrenia	**1.61E-10**	**9.69E-05**	**2.04E-06**	**4.70E-04**
Anxiety + Depression	**4.94E-12**	**4.19E-05**	**5.33E-08**	**7.93E-05**
Schizophrenia + Bipolar	**2.56E-12**	**6.00E-06**	**5.84E-08**	**3.07E-05**
All psychiatric drugs	**1.23E-16**	**9.29E-07**	**2.94E-10**	**4.86E-06**

Self: self-contained test; Compet, competitive test. Test results with q-value < 0.05 are in bold. Results with q-value between 0.05 and 0.1 are in italics. Full tables of q-values are presented in Supplementary Table 3.

Table 4 Selected repositioning hits based on meta-analyzed p-values (Brown's or Simes' method)

Drug	pval_Simes	pval_Brown	qval_Simes	qval_Brown	Drug Description	Reference
Fendiline	1.21E-10	1.06E-11	6.33E-07	5.55E-08	Nonselective calcium channel blocker; produce antidepressant-like effects in mouse models by inhibition of acid sphingomyelinase and reduction of ceramide levels	[35]
Alsterpaullone	1.01E-06	1.25E-04	1.32E-03	2.97E-02	Competitive inhibitor of GSK-3β; GSK3 inhibition is implicated in various psychiatric disorders, including depression	[36,37]
Sanguinarine	6.05E-04	1.56E-04	7.19E-02	3.02E-02	A selective mitogen-activated protein kinase phosphatase-1 (Mkp-1) inhibitor; shown to produce antidepressant-like effect in rats	[41]
Piperlongumine	1.17E-04	1.45E-03	2.45E-02	1.48E-01	A constituent of *Piper longum* fruit; shown to confer resistance against stress in a mouse model	[42]

Pval, p-values; qval, q-values. Please refer to the main text for more detailed discussions.

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References:

1. World Health Organization. Depression and Other Common Mental Disorders Global Health Estimates. Available at http://apps.who.int/iris/bitstream/10665/254610/1/WHO-MSD-MER-2017.2-eng.pdf?ua=1. (2017).
2. Frances, A. *et al.* Relationship of anxiety and depression. *Psychopharmacology (Berl)* **106 Suppl**, S82-6 (1992).
3. Johansson, R., Carlbring, P., Heedman, A., Paxling, B. & Andersson, G. Depression, anxiety and their comorbidity in the Swedish general population: point prevalence and the effect on health-related quality of life. *PeerJ* **1**, e98 (2013).
4. Trivedi, M.H. *et al.* Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. *American Journal of Psychiatry* **163**, 28-40 (2006).
5. Al-Harbi, K.S. Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient Preference and Adherence* **6**, 369-388 (2012).
6. Papassotiropoulos, A. & de Quervain, D.J.F. Failed drug discovery in psychiatry: time for human genome-guided solutions. *Trends in Cognitive Sciences* **19**, 183-187 (2015).
7. Novac, N. Challenges and opportunities of drug repositioning. *Trends in Pharmacological Sciences* **34**, 267-272 (2013).
8. Sanseau, P. *et al.* Use of genome-wide association studies for drug repositioning. *Nat Biotechnol* **30**, 317-20 (2012).
9. Anighoro, A., Bajorath, J. & Rastelli, G. Polypharmacology: Challenges and Opportunities in Drug Discovery. *Journal of Medicinal Chemistry* **57**, 7874-7887 (2014).
10. So, H.-C. *et al.* When GWAS meets the Connectivity Map: drug repositioning for seven psychiatric disorders. *bioRxiv*, 096503 (2016).
11. Maciejewski, H. Gene set analysis methods: statistical models and methodological differences. *Briefings in Bioinformatics* **15**, 504-518 (2014).
12. de Leeuw, C.A., Neale, B.M., Heskes, T. & Posthuma, D. The statistical properties of gene-set analysis. *Nature Reviews Genetics* **17**, 353-364 (2016).
13. de Jong, S., Vidler, L.R., Mokrab, Y., Collier, D.A. & Breen, G. Gene-set analysis based on the pharmacological profiles of drugs to identify repurposing opportunities in schizophrenia. *J Psychopharmacol* **30**, 826-30 (2016).
14. Gaspar, H.A. & Breen, G. Pathways analyses of schizophrenia GWAS focusing on known and novel drug targets. *bioRxiv*, 091264 (2016).
15. Sullivan, P.F. *et al.* A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular Psychiatry* **18**, 497-511 (2013).
16. Cai, N. *et al.* Sparse whole-genome sequencing identifies two loci for major depressive disorder. *Nature* **523**, 588-+ (2015).
17. Okbay, A. *et al.* Genetic variants associated with subjective well-being, depressive symptoms, and neuroticism identified through genome-wide analyses (vol 48, pg 624, 2016). *Nature Genetics* **48**, 1591-1591 (2016).
18. Lahey, B.B. Public Health Significance of Neuroticism. *American Psychologist* **64**, 241-256 (2009).
19. Tang, T.Z. *et al.* Personality Change During Depression Treatment A Placebo-Controlled Trial. *Archives of General Psychiatry* **66**, 1322-1330 (2009).
20. Otowa, T. *et al.* Meta-analysis of genome-wide association studies of anxiety disorders. *Molecular Psychiatry* **21**, 1391-1399 (2016).
21. Yoo, M. *et al.* DSigDB: drug signatures database for gene set analysis. *Bioinformatics* **31**, 3069-3071 (2015).

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22. Kim, S. *et al.* PubChem Substance and Compound databases. *Nucleic Acids Research* **44**, D1202-D1213 (2016).
23. Gaulton, A. *et al.* ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Research* **40**, D1100-D1107 (2012).
24. Lamb, J. *et al.* The connectivity map: Using gene-expression signatures to connect small molecules, genes, and disease. *Science* **313**, 1929-1935 (2006).
25. Mattingly, C.J., Colby, G.T., Forrest, J.N. & Boyer, J.L. The Comparative Toxicogenomics Database (CTD). *Environmental Health Perspectives* **111**, 793-795 (2003).
26. Bakshi, A. *et al.* Fast set-based association analysis using summary data from GWAS identifies novel gene loci for human complex traits. *Scientific Reports* **6**(2016).
27. Liu, J.Z. *et al.* A Versatile Gene-Based Test for Genome-wide Association Studies. *American Journal of Human Genetics* **87**, 139-145 (2010).
28. Purcell, S. *et al.* PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* **81**, 559-575 (2007).
29. de Leeuw, C.A., Mooij, J.M., Heskes, T. & Posthuma, D. MAGMA: Generalized Gene-Set Analysis of GWAS Data. *Plos Computational Biology* **11**(2015).
30. Simes, R.J. An improved Bonferroni procedure for multiple tests of significance. *Biometrika*, 751-754 (1986).
31. Brown, M.B. 400: A method for combining non-independent, one-sided tests of significance. *Biometrics*, 987-992 (1975).
32. Sarkar, S.K. & Chang, C.-K. The Simes method for multiple hypothesis testing with positively dependent test statistics. *Journal of the American Statistical Association* **92**, 1601-1608 (1997).
33. Wei, W.Q. *et al.* Development and evaluation of an ensemble resource linking medications to their indications. *J Am Med Inform Assoc* **20**, 954-61 (2013).
34. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Methodological* **57**, 289-300 (1995).
35. Gulbins, E. *et al.* Acid sphingomyelinase-ceramide system mediates effects of antidepressant drugs. *Nature Medicine* **19**, 934-+ (2013).
36. Leost, M. *et al.* Paullones are potent inhibitors of glycogen synthase kinase-3 beta and cyclin-dependent kinase 5/p25. *European Journal of Biochemistry* **267**, 5983-5994 (2000).
37. Jope, R.S. & Roh, M.S. Glycogen synthase kinase-3 (GSK3) in psychiatric diseases and therapeutic interventions. *Current Drug Targets* **7**, 1421-1434 (2006).
38. Diniz, B.S. *et al.* Platelet GSK3B activity in patients with late-life depression: Marker of depressive episode severity and cognitive impairment? *World Journal of Biological Psychiatry* **12**, 216-222 (2011).
39. Beaulieu, J.M. *et al.* Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 1333-1338 (2008).
40. Freland, L. & Beaulieu, J.M. Inhibition of GSK3 by lithium, from single molecules to signaling networks. *Frontiers in Molecular Neuroscience* **5**(2012).
41. Chen, Y.C. *et al.* Microinjection of sanguinarine into the ventrolateral orbital cortex inhibits Mkp-1 and exerts an antidepressant-like effect in rats. *Neuroscience Letters* **506**, 327-331 (2012).
42. Yadav, V., Chatterjee, S.S., Majeed, M. & Kumar, V. Long lasting preventive effects of piperlongumine and a Piper longum extract against stress triggered pathologies in mice. *Journal of Intercultural Ethnopharmacology* **4**, 277-283 (2015).

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43. Fung, M. Evaluation of the characteristics of safety withdrawal of prescription drugs from worldwide pharmaceutical markets-1960 to 1999. *Drug Information Journal* **35**, 293-317 (2001).
44. Cipriani, A. *et al.* A systematic review of calcium channel antagonists in bipolar disorder and some considerations for their future development. *Molecular Psychiatry* **21**, 1324-1332 (2016).
45. Ostacher, M.J. *et al.* Pilot investigation of isradipine in the treatment of bipolar depression motivated by genome-wide association. *Bipolar Disorders* **16**, 199-203 (2014).
46. Dubovsky, S.L., Buzan, R., Thomas, M., Kassner, C. & Cullum, C.M. Nicardipine improves the antidepressant action of ECT but does not improve cognition. *J ECT* **17**, 3-10 (2001).
47. Taragano, F.E., Allegri, R., Vicario, A., Bagnatti, P. & Lyketsos, C.G. A double blind, randomized clinical trial assessing the efficacy and safety of augmenting standard antidepressant therapy with nimodipine in the treatment of 'vascular depression'. *International Journal of Geriatric Psychiatry* **16**, 254-260 (2001).
48. Taragano, F.E., Bagnatti, P. & Allegri, R.F. A double-blind, randomized clinical trial to assess the augmentation with nimodipine of antidepressant therapy in the treatment of "vascular depression". *International Psychogeriatrics* **17**, 487-498 (2005).
49. Balon, R. & Ramesh, C. Calcium channel blockers for anxiety disorders? *Ann Clin Psychiatry* **8**, 215-20 (1996).
50. Goldberg, D. The heterogeneity of "major depression". *World Psychiatry* **10**, 226-8 (2011).
51. Fournier, J.C. *et al.* Antidepressant drug effects and depression severity: a patient-level meta-analysis. *JAMA* **303**, 47-53 (2010).
52. Kirsch, I. *et al.* Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. *PLoS Med* **5**, e45 (2008).
53. Spielmans, G.I. *et al.* Adjunctive atypical antipsychotic treatment for major depressive disorder: a meta-analysis of depression, quality of life, and safety outcomes. *PLoS Med* **10**, e1001403 (2013).
54. Zhou, X.Y. *et al.* Atypical Antipsychotic Augmentation for Treatment-Resistant Depression: A Systematic Review and Network Meta-Analysis. *International Journal of Neuropsychopharmacology* **18**(2015).
55. Gao, K., Muzina, D., Gajwani, P. & Calabrese, J.R. Efficacy of typical and atypical antipsychotics for primary and comorbid anxiety symptoms or disorders: a review. *J Clin Psychiatry* **67**, 1327-40 (2006).
56. Samuel, M., Zimovetz, E.A., Gabriel, Z. & Beard, S.M. Efficacy and safety of treatments for refractory generalized anxiety disorder: a systematic review. *Int Clin Psychopharmacol* **26**, 63-8 (2011).
57. LaLonde, C.D. & Van Lieshout, R.J. Treating generalized anxiety disorder with second generation antipsychotics: a systematic review and meta-analysis. *J Clin Psychopharmacol* **31**, 326-33 (2011).
58. Cross-Disorder Group of the Psychiatric Genomics, C. *et al.* Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet* **45**, 984-94 (2013).
59. Smith, D.J. *et al.* Genome-wide analysis of over 106000 individuals identifies 9 neuroticism-associated loci. *Molecular Psychiatry* **21**, 749-757 (2016).