Genetic variation associated with euphorogenic effects of d-amphetamine is associated with diminished risk for schizophrenia and attention deficit hyperactivity disorder

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Here, we extended our findings from a genome-wide association study of the euphoric response to d-amphetamine in healthy human volunteers by identifying enrichment between SNPs associated with response to d-amphetamine and SNPs associated with psychiatric disorders. We found that SNPs nominally associated (P ≤ 0.05 and P ≤ 0.01) with schizophrenia and attention deficit hyperactivity disorder were also nominally associated with d-amphetamine response. Furthermore, we found that the source of this enrichment was an excess of alleles that increased sensitivity to the euphoric effects of d-amphetamine and increased susceptibility to schizophrenia and attention deficit hyperactivity disorder. In contrast, three negative control phenotypes (height, inflammatory bowel disease, and Parkinson disease) did not show this enrichment. Taken together, our results suggest that alleles identified using an acute challenge with a dopaminergic drug in healthy individuals can be used to identify alleles that confer risk for psychiatric disorders commonly treated with dopaminergic agonists and antagonists. More importantly, our results show the use of the enrichment approach as an alternative to stringent standards for genome-wide significance and suggest a relatively novel approach to the analysis of small cohorts in which intermediate phenotypes have been measured.

Significance

We show that the genetic susceptibility to the euphoric effects of d-amphetamine also influences the genetic predisposition to schizophrenia and attention deficit hyperactivity disorder (ADHD). These results reinforce the idea that dopamine plays a role in schizophrenia and ADHD; this so-called dopamine hypothesis has been debated for several decades. Specifically, we found that the alleles associated with increased euphoric response to d-amphetamine were associated with decreased risk for schizophrenia and ADHD. These results illustrate how an acute challenge with a pharmacological agent can reveal a genetic predisposition that will manifest itself as psychiatric illness over the lifetime of an individual. Finally, our study offers a relatively novel paradigm for the analysis of endophenotypes for which large sample sizes are not typically available.
we found a significant enrichment of schizophrenia-associated SNPs among the SNPs associated with amphetamine response at both the \( P \leq 0.01 \) and \( P \leq 0.05 \) thresholds (empirical \( P = 0.007 \) and \( P = 0.033 \), respectively) (Fig. 2A).

We hypothesized that, if the enrichment phenomena were based on a real biological phenomenon, there would be a consistent relationship between the direction of the effect (positive or negative) of alleles on risk for schizophrenia and sensitivity to the euphoric effects of amphetamine. To test this hypothesis, we performed two analyses: one analysis in which alleles that increased the risk for schizophrenia also increased amphetamine response (concordant) and one analysis in which alleles that increased the risk for schizophrenia decreased amphetamine response (discordant). This analysis could not be performed in the GAIN schizophrenia study, because odds ratios were unavailable. In the PGC1 schizophrenia dataset, we found that 239 of 380 SNPs (62.9\%) that constituted the enriched set at the PGC1 schizophrenia sample. A schematic representation of the enrichment analysis is shown in Left. There was a significant enrichment of SNPs nominally associated with schizophrenia among SNPs nominally associated with the euphoric response to d-amphetamine; the enrichment was significant with \( P \) value thresholds of (Center) \( P \leq 0.01 \) and (Right) \( P \leq 0.05 \). The black dots represent the number of overlapping SNPs. The histograms represent the null distribution of overlapping SNPs generated from 1,000 random permutations of the amphetamine data. SCZ, schizophrenia. *\( P < 0.05 \).

SNPs Associated with the Euphoric Response to d-Amphetamine Are Enriched for SNPs Associated with Protection from Attention Deficit Hyperactivity Disorder. We observed significant enrichment of attention deficit hyperactivity disorder (ADHD)-associated SNPs among the SNPs associated with amphetamine response at both the \( P \leq 0.01 \) and \( P \leq 0.05 \) thresholds (empirical \( P = 0.017 \) for discordant SNPs and empirical \( P = 0.440 \) for concordant SNPs) (Fig. 2B). Therefore, the significant enrichment of schizophrenia-associated SNPs among amphetamine-associated SNPs was driven by discordant alleles.
were associated with increased amphetamine response. Similar results were observed at the \( P \leq 0.05 \) threshold (empirical \( P = 0.038 \) for discordant SNPs and empirical \( P = 0.394 \) for concordant SNPs) (Fig. 3B).

**SNPs Associated with the Euphoric Response to d-Amphetamine Are Not Enriched for SNPs Associated with Three Negative Control Phenotypes.** We considered the possibility that enrichment might be caused by linkage disequilibrium (LD) structure or some unexpected artifact not properly accounted for by the permutation analysis and thus, would be observed in any large GWAS. To evaluate this possibility, we examined enrichment in three negative control phenotypes for which large samples were available. We found no significant enrichment of SNPs associated with height at the \( P \leq 0.01 \) or \( P \leq 0.05 \) thresholds (Fig. 4A) (\( P = 0.518 \) and \( P = 0.441 \), respectively). Similarly, there was no significant enrichment of SNPs associated with inflammatory bowel disease at the \( P \leq 0.01 \) threshold (Fig. 4B) (empirical \( P = 0.391 \)); data for inflammatory bowel disease at the \( P \leq 0.05 \) threshold were not available. Additionally, we saw no enrichment for Parkinson disease-associated SNPs at either the \( P \leq 0.01 \) or \( P \leq 0.05 \) thresholds (Fig. 4C) (\( P = 0.126 \) and \( P = 0.836 \), respectively). In terms of directionality in the negative control samples, we found no significant enrichment of concordant or discordant SNPs in the Parkinson disease dataset. We were unable to obtain directional information for the height and inflammatory bowel disease datasets. However, we were able to obtain directional information for a Crohn disease GWAS dataset that largely overlaps with a subset of the inflammatory bowel disease sample (9). Using that dataset, we observed no significant overall enrichment and no significant enrichment of concordant or discordant SNPs.

**Similar Results Are Observed When Imputed SNPs from the Amphetamine Response Dataset Are Excluded.** All results presented were derived from analyses using amphetamine response data that consist of a mixture of directly genotyped and imputed SNPs. To assess the possibility that an artifact related to imputation had caused the observed enrichment, we conducted similar analyses that were restricted to directly genotyped SNPs in the amphetamine response dataset; these results were not meaningfully different (Fig. S1). Thus, these results do not seem to be an artifact of imputation.

**Enrichment of Schizophrenia and ADHD-Associated SNPs Is Observed in Replication Samples.** To replicate our findings of enrichment for schizophrenia-associated SNPs in the GAIN and PGC1 datasets, we obtained an additional replication dataset [Swedish schizophrenia sample (10)] and repeated our analyses in the replication sample alone and the combined meta-analysis sample (PGC1 schizophrenia + Swedish schizophrenia). When considering only the Swedish schizophrenia sample, we observed borderline significant enrichment at the \( P \leq 0.05 \) threshold (\( P = 0.067 \)); when we performed the same analysis in the meta-analysis sample (PGC1 schizophrenia + Swedish schizophrenia), we found that the strength of enrichment improved (\( P = 0.021 \)) compared with the same analysis in the PGC1 schizophrenia sample alone. We also found that the strength of enrichment among the discordant SNPs was slightly improved in this larger meta-analysis sample (\( P = 0.016 \)) compared with the results from the PGC1 schizophrenia data.

Similarly, we were able to replicate our findings in a newer ADHD replication dataset [Psychiatric Genomics Consortium phase 2 (PGC2 ADHD) using the \( P \leq 0.05 \) threshold. In this case, we did not observe a significant enrichment when using only the ADHD replication dataset (PGC2 ADHD); however, we did observe a nearly significant enrichment of discordant direction SNPs (\( P = 0.060 \)). Similarly, in the meta-analysis sample (PGC1 ADHD + PGC2 ADHD), we observed an even more significant enrichment of discordant direction SNPs (\( P = 0.010 \)) in the meta-analysis sample compared with the PGC1 ADHD sample alone.

**SNPs Associated with the Increased Euphoric Response to d-Amphetamine Are Enriched for SNPs That Confer Protection from Bipolar Disorder.** We hypothesized that SNPs associated with the euphoric response to amphetamine may also be enriched for SNPs
associated with bipolar disorder. We did not observe an overall significant enrichment (Fig. S2). However, when we stratified SNPs by concordant vs. discordant, we again observed a significant enrichment of discordant SNPs at both the $P \leq 0.01$ and $P \leq 0.05$ thresholds (empirical $P = 0.018$ and $P = 0.045$, respectively) (Fig. S2).

A Subset of the SNPs That Are Associated with the Euphoric Response to $d$-Amphetamine Are Enriched for SNPs That Confer Protection from Both Schizophrenia and ADHD. We were interested in testing whether any of the SNPs that overlapped with $d$-amphetamine response were shared with both schizophrenia and ADHD. Shared SNPs would suggest shared biology, potentially related to dopaminergic function. We found significant enrichment for discordant SNPs among associations with $d$-amphetamine response. The histograms represent the null distribution of overlapping SNPs generated from 1,000 random permutations of the amphetamine data. A shows the results for the height enrichment analysis. Results from the $P \leq 0.01$ threshold are shown in Left, and results from the $P \leq 0.05$ threshold are shown in Right. The black dots represent the observed count of height-associated SNPs among associations with $d$-amphetamine response. The histogram represents the null distribution of overlapping SNPs generated from 1,000 random permutations of the amphetamine data. None of these results were significant. C shows the results for the Parkinson disease enrichment analysis. Results from the $P \leq 0.01$ threshold are shown in Left, and results from the $P \leq 0.05$ threshold are shown in Right. The black dots represent the observed count of Parkinson disease-associated SNPs among associations with $d$-amphetamine response. The histograms represent the null distribution of overlapping SNPs generated from 1,000 random permutations of the amphetamine data. GIANT, Genetic Investigation of Anthropometric Traits.

Discussion
Our results show that SNPs associated with response to a dopaminergic drug challenge ($d$-amphetamine) are enriched for SNPs associated with psychiatric disorders that are treated with dopamine agonists (ADHD) and antagonists (schizophrenia). Rather than identifying a few SNPs with a high degree of statistical confidence, our method is intended to identify a heterogeneous collection of SNPs that is made up of both true- and false-positive associations. We show that this enrichment was caused by alleles that increased the euphoric response to amphetamine and decreased the risk for both schizophrenia and ADHD. In contrast, no enrichment was observed for concordant SNPs or any non-psychiatric phenotypes. We also showed that the results were not an artifact of imputation and that these effects could be replicated in multiple samples.

Of the theories regarding the underlying mechanisms for schizophrenia, the so-called dopamine hypothesis has been the most enduring (11, 12). Although this theory is still under debate (13, 14), several lines of evidence lend credence to the hypothesis. For example, the efficacy of typical antipsychotic drugs is almost linearly related to their affinity for the dopamine D2 receptor (15). Additionally, when high doses of amphetamine are ingested for a protracted period, psychotic symptoms can develop (16). Several studies have shown increased striatal dopamine release in response to a $d$-amphetamine challenge in schizophrenics and consequently, a worsening of symptoms (17, 18). Our study adds genetic evidence to support the dopaminergic hypothesis of schizophrenia using a cohort of healthy volunteers carefully screened against Axis I psychiatric disorders. A dopamine hypothesis of ADHD has also been proposed and challenged (19, 20). ADHD is often treated with methylphenidate or amphetamine products ($d$-amphetamine, mixed amphetamine salts, or lisdexamfetamine) (21). The therapeutic effects of these drugs are believed to be caused by their ability to increase the synaptic availability of dopamine. Interestingly, our results suggest that insensitivity to a drug that is used to treat ADHD might be a genetic risk factor for ADHD; however, it is important to note that we examined sensitivity to the euphoric effects of amphetamine and not sensitivity to its therapeutic effects. Our results are consistent with studies that have shown a protective effect from substance use disorders in stimulant-treated adolescents with ADHD (22, 23).

A puzzling feature of our results is that we saw enrichment of protective alleles for both schizophrenia and ADHD among our top associations with acute amphetamine response, whereas a simplistic understanding of these disorders suggests different types of dopamine dysregulation: excess dopamine in schizophrenia vs. dopamine deficit in ADHD. There is mixed evidence for shared genetic risk for schizophrenia and ADHD. A higher incidence of ADHD symptoms has been observed among relatives of schizophrenic patients compared with healthy controls (24) as well as increased risk for schizophrenia among relatives of individuals with ADHD (25). A recent polygenic risk score analysis identified shared genetic susceptibility between schizophrenia and ADHD (26). However, another recent study did not identify
significant polygenic risk overlap for schizophrenia and ADHD (6), and a different recent study found no significant genetic correlation estimated from SNP heritabilities for the two disorders (27). Our approach is different, because we are examining only the subset of SNPs that is associated with both amphetamine response and these psychiatric disorders, which may explain the discrepancy between our results and these two recent studies and may identify another advantage of our approach.

These data suggest that our acute amphetamine response phenotype may be viewed as an endophenotype for schizophrenia and ADHD. Whereas prior definitions of endophenotypes have focused on cosegregation of the putative endophenotype and the disease phenotype, we examined associations at SNPs throughout the genome to establish a genetic link between amphetamine response with both schizophrenia and ADHD. Our sample was specifically screened to exclude individuals with Axis I disorders, which should have depleted the number of risk alleles present in this population. The results suggest a relatively novel approach to the empirical validation of endophenotypes.

Comorbidity of ADHD and bipolar disorder has been reported in the literature (28), and thus, we considered the possibility of enrichment of bipolar disorder-associated SNPs and amphetamine response-associated SNPs. Although we did not observe overall enrichment, we did observe directionality, with significant enrichment of discordant SNPs at this P value thresholds. These results suggest that, in addition to schizophrenia and ADHD, the acute amphetamine response phenotype may also be an endophenotype for bipolar disorder (29).

We initially conceived of the acute response to amphetamine as an intermediate phenotype for drug abuse. However, our results suggest that acute drug challenge phenotypes may be useful in identifying SNPs that are functionally relevant to psychiatric disorders. Based on this study, it may be reasonable to ask whether sensitivity to therapeutic drugs (or drugs that cause worsening of symptoms) may uncover alleles that influence risk or protection for other disorders. Whether acute amphetamine response is indeed a useful intermediate phenotype for drug abuse or other disorders may be determined in future studies; related research examining the euphoric response to alcohol has proven fruitful (30–34).

Our amphetamine response GWAS was based on a relatively small sample. Lack of power is likely to contribute to the inability to achieve signals that survive multiple testing corrections in the GWAS of psychiatric phenotypes (35). By taking an enrichment approach, we were able to capitalize on associations that did not meet stringent genome-wide significance criteria but were nominally associated with amphetamine response. Our results suggest that the enrichment approach is complementary to the traditional GWAS approach and a valuable secondary analysis. In contrast to GWAS, which aims to identify specific SNPs, the power of our method is that it can draw biological inferences from a heterogeneous set of SNPs composed of both true and false positives. However, this method is unable to distinguish between these two categories.

Although our study is not without limitations, we considered several alternative explanations for our observations, but none proved credible. One possibility was that results from any two GWAS may overlap because of LD patterns. By using permutation, we preserved the LD structure among the SNPs being tested, which should guard against such a phenomenon. This possibility is further addressed by the directional analyses and our use of negative control phenotypes. We considered the possibility that the enrichment that we observed was driven by functional brain SNPs (e.g., expression quantitative trait loci) that would be enriched for any brain disease. However, we saw no enrichment for Parkinson disease-associated SNPs, suggesting that our results are specific to schizophrenia and ADHD; the results from our directional analyses of schizophrenia and ADHD further dispute the possibility that the overlapping SNPs are important for all brain diseases. We were also concerned that artifacts caused by imputation could bias our results. However, we observed similar results when we considered only SNPs that were directly genotyped in the amphetamine response sample; permutation should further guard against any such artifacts (Fig. S1). Our results are further strengthened by the fact that they were observed in multiple datasets.

By examining our GWAS results through the lens of enrichment, we were able to interrogate results that do not meet stringent criteria for statistical significance. Our results suggest that alleles identified using an acute drug challenge can be used to identify alleles that influence risk for psychiatric disorders. Our results also support the dopamine hypotheses of schizophrenia and ADHD. Ultimately, this study shows that additional secondary analyses of GWAS results may provide new insights into the biology of psychiatric disorders. These results also suggest a useful and generalizable method for the genetic analysis of modestly sized intermediate phenotypes that are unlikely to yield genome-wide significant results and for which replication samples are not typically available.

Materials and Methods

Genetics of Amphetamine Dataset. Study details are provided in the work by Hart et al. (7). This study was approved by the Institutional Review Board of The University of Chicago and was carried out in accordance with the Helsinki Declaration of 1975. Briefly, 381 healthy volunteers attended three separate test sessions, during which they received d-amphetamine (placebo, 10 mg, or 20 mg) under double blind conditions and subjective self-report questionnaires at regular intervals: the Profile of Mood States (36), Drug Effects Questionnaire (37), and Addiction Research Center Inventory (38). Sparse factor analysis (39) was used to reduce the dimensionality of the phenotype data to a small number of factors that explained both drug response and baseline characteristics of the sample. For the present study, we limited our analyses to the 10%-d-amphetamine] response factor. This factor, hereafter referred to as amphetamine response, was one of the most interpretable factors, reflecting the subjective euphoric response to amphetamine, and it showed the strongest association signal (7). Subjects were genotyped using Affymetrix 6.0 arrays. Imputation was performed using the HapMap3 and 1000 Genomes reference panels (40, 41). Self-reported ancestry was confirmed by analysis with the Admixture software package (42). The sample used in the current study was restricted to participants of European ancestry (n = 325). After quality control and imputation, 5,974,669 SNPs were available for analysis. The samples used for the enrichment analysis are shown in Table S1; additional details are given in the SI Materials and Methods.

Data Preparation. In the Genetics of Amphetamine dataset, SNPs with minor allele frequencies < 0.01 were removed. Genotypes were converted into PLINK format with GTOOL (www.well.ox.ac.uk/~c finesm an/software/gwas/g tool .html) with a threshold of 0.8 specified; markers with missing rates > 10% were excluded. The amphetamine response phenotype was permuted 1,000 times using the “make-perm-pheno” command in PLINK (43), and association testing was run with each of these 1,000 permuted phenotypes with the PLINK “assoc” command. The numbers of SNPs available for the enrichment analysis are listed in Table S2.

Enrichment Analysis. The number of SNPs that overlapped between the amphetamine response results and the results for each of the phenotypes described above was recorded (for both the P ≤ 0.01 and P ≤ 0.05 thresholds). Next, the number of overlapping SNPs in each permuted dataset (n = 1,000) was recorded, yielding the expected null distribution. The empirical P value was computed as the fraction of permutations where the number of overlapping SNPs matched or exceeded the observed count. A statistically significant enrichment was defined as an enrichment P value < 0.05 (i.e., less than 50 permutations were found with a greater number of overlapping SNPs).

Directionality Analysis. For the SNPs that overlapped between the phenotypes examined in the enrichment analyses described above, we examined the direction of the effect in both the amphetamine response and the second phenotype. The signs of the logistic regression β-coefficients [i.e., ln(odd ratio)] were used to denote directionality. The Z scores from the PGC1 ADHD results were used to denote directionality of the association, with Z score > 0 corresponding to odds ratio > 1. The signs of the β-coefficients or Z scores were flipped if the PGC reference allele did not match the reference allele in the amphetamine response dataset. We recorded the number of concordant SNPs (positive in both samples or negative in both samples) and the number of discordant SNPs (positive in one sample and negative in the other sample) in the real and permuted datasets. This procedure generated the expected
null distribution of concordant alleles (e.g., alleles associated with risk as well as heightened response to amphetamine) and the expected null distribution of discordant alleles. Excluding strand ambiguous SNPs had no effect on our results. The empirical P value was computed as the proportion of permutations where the number of overlapping SNPs matched or exceeded the count observed in the real data.

Replication Analyses. Enrichment and directionality analyses were performed as described above in the replication samples alone (Swedish schizophrenia study and PGC2 ADHD) and the combined meta-analysis samples (PGC1 schizophrenia + Swedish schizophrenia and PGC1 ADHD + PGC2 ADHD). Meta-analysis was performed with the “meta-analysis” command in PLINK.


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Supporting Information

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SI Materials and Methods

Enrichment Datasets. GAIN Schizophrenia. This GWAS (1) included 1,351 European-American cases with schizophrenia and 1,378 European-American controls. Subjects were genotyped on the Affymetrix 6.0 array. After quality control, 729,454 SNPs were available for analysis. Precomputed association results were obtained from dbGaP (phs000021.v3.p2). This sample is a subset of the PGC1 schizophrenia sample (below).

PGC1 Schizophrenia. This mega-analysis (2) included 9,394 European cases and 12,462 European controls. Imputation was performed with the HapMap3 reference panel (3). After quality control, 1,252,902 SNPs were available for analysis. Publicly available association results were obtained from https://pgc.unc.edu/Sharing.php.

PGC1 ADHD. This meta-analysis (4) included four separate ADHD studies, with the final dataset comprised of 2,064 trios, 896 cases, and 2,455 controls of European ancestry. Imputation was performed with the HapMap3 reference panel (3). After quality control, 1,206,462 SNPs were available for analysis. Publicly available association results were obtained from https://pgc.unc.edu/Sharing.php.

PGC1 Bipolar Disorder. This GWAS (5) included 7,481 European cases and 9,250 European controls. Imputation was performed with the HapMap2 reference panel (6). After quality control, 2,541,952 SNPs were available for analysis. Publicly available association results were obtained from https://pgc.unc.edu/Sharing.php.

Negative control phenotype: Genetic Investigation of Anthropometric Traits Height. This meta-analysis (7) included 133,653 European individuals. Imputation was performed with the HapMap2 reference panel (6). After quality control, 2,469,636 SNPs were available for analysis. Publicly available association results were obtained from www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files.

Negative control phenotype: International Inflammatory Bowel Disease Genetics Consortium Inflammatory Bowel Disease. This GWAS (8) included 12,882 European inflammatory bowel disease (Crohn disease and ulcerative colitis) cases and 21,770 European controls. Imputation was performed with the HapMap3 reference panel (3). After quality control, 1,252,901 SNPs were available for analysis. Publicly available association results were obtained from www.ibdgenetics.org/downloads.html. Available results were restricted to $P \leq 0.01$; thus, we could not examine enrichment at the $P \leq 0.05$ threshold.

Negative control phenotype: Parkinson Disease GWAS Consortium Parkinson Disease. Study details are provided in the work by Pankratz et al. (9). The meta-analysis consisted of 4,238 European Parkinson disease cases and 4,239 European controls. Imputation was performed with the HapMap2 reference panel (6). After quality control, 2,525,705 SNPs were available for analysis. Full association results were obtained from ref. 9.

Replication Datasets. Swedish Schizophrenia Study. This GWAS (10) included 5,001 schizophrenia cases and 6,243 controls from a population-based sampling frame in Sweden ($n = 11,244$). Samples were genotyped in six batches using Affymetrix 5.0 (3.9%), Affymetrix 6.0 (38.6%), and Illumina OmniExpress (57.4%) chips. After quality control and imputation with the 1000 Genomes Project Phase 1 reference panel, we analyzed association result from allelic dosages for 9,871,789 high-quality polymorphic SNPs.

PGC2 ADHD. This meta-analysis included data from a total of nine cohorts [Cardiff: 641 cases and 1,752 controls; Chinese: 1,012 cases and 930 controls; Germany: 494 cases and 1,297 controls; International Multicenter ADHD Genetics project phase 2 (IMAGE2): 787 cases and 7,082 controls; Spain: 591 cases and 432 controls; Children’s Hospital of Philadelphia (CHOP): 358 trios; Canada: 170 trios; International Multicenter ADHD Genetics project phase 1 (IMAGE1): 866 trios; Pfizer-funded study from the University of California, Los Angeles, Washington University, and the Massachusetts General Hospital (PUWMA): 702 trios]. The IMAGE1, IMAGE2, PUWMA, and CHOP trios constituted the PGC1 set described in the work by Neale et al. (4). The Cardiff, Chinese, German, Spain, and Canada cohorts constituted the independent replication sample. Imputation was performed with the HapMap3 reference panel (3). After quality control, 1,384,810 SNPs were available for analysis.

Fig. S1. Enrichment results for analyses limited to SNPs that were directly genotyped in the amphetamine response dataset. Significant enrichment is seen for schizophrenia- and attention deficit hyperactivity disorder (ADHD)-associated SNPs among amphetamine response associations computed with directly genotyped SNPs (nonimputed). The black dots represent the observed count of trait-associated SNPs among associations with d-amphetamine response. The histograms represent the number of SNPs that occurred among association results from 1,000 random permutations. GIANT, Genetic Investigation of Anthropometric Traits; IBD, inflammatory bowel disease; IIBDGC, International Inflammatory Bowel Disease Genetics Consortium; PGC1, Psychiatric Genomics Consortium phase 1; SCZ, schizophrenia. *P < 0.05.
SNPs associated with the euphoric response to d-amphetamine are enriched among SNPs associated with protection from bipolar disorder. A shows a schematic representation of the enrichment analysis. There was no significant enrichment of SNPs that were nominally associated with bipolar disorder from the PGC1 bipolar disorder sample among SNPs nominally associated with the euphoric response to d-amphetamine at either P value threshold. The black dots represent the observed number of overlapping SNPs. The histograms represent the null distribution of overlapping SNPs generated from 1,000 random permutations of the amphetamine data. B shows the same analysis as A, except that SNPs were only considered if they were concordant (Upper) or discordant (Lower) in direction. These results indicate an enrichment for discordant SNPs. AMPH, d-amphetamine; PGC1, Psychiatric Genomics Consortium phase 1. *P < 0.05.
SNPs associated with the euphoric response to amphetamine overlap with SNPs associated with decreased risk for schizophrenia and decreased risk for ADHD. We examined the SNPs that were overlapping between the three dopaminergic phenotypes: euphoric response to d-amphetamine, schizophrenia, and ADHD. A shows the results for the overall nominally significant enrichment for SNPs that overlap between the three phenotypes ($P = 0.062$). (A and B) The red dots represent the observed number of overlapping SNPs. The histograms represent the null distribution of overlapping SNPs generated from 1,000 random permutations of the amphetamine data. B shows the results for the concordant SNPs (SNPs associated with increased euphoria and increased schizophrenia and ADHD risk) and the discordant SNPs (SNPs associated with increased euphoria and decreased risk for schizophrenia and ADHD). We only observed enrichment for the discordant SNPs. AMPH, d-amphetamine; PGC1, Psychiatric Genomics Consortium phase 1; SCZ, schizophrenia. *$P < 0.05$. 
Table S1. Description of enrichment samples

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<th>Sample</th>
<th>Ref.</th>
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<tr>
<td>PGC1 ADHD</td>
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<td>896 cases; 2,455 controls; 2,064 trios</td>
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<td>PGC1 Bipolar Disorder</td>
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<td>Genetic Investigation of Anthropometric Traits Height</td>
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GWAS, genome-wide association study; IIBDGC, International Inflammatory Bowel Disease Genetics Consortium; PGC1, Psychiatric Genomics Consortium phase 1; PGC2, Psychiatric Genomics Consortium phase 2.


Table S2. Numbers of SNPs available from each dataset for the enrichment analysis

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<tr>
<td>PGC1 ADHD + PGC2 ADHD meta-analysis</td>
<td>15,527</td>
<td>14,228</td>
</tr>
<tr>
<td>PGC1 Bipolar Disorder</td>
<td>43,729</td>
<td>40,415</td>
</tr>
<tr>
<td>Genetic Investigation of Anthropometric Traits Height</td>
<td>72,893</td>
<td>66,200</td>
</tr>
<tr>
<td>IIBDGC Inflammatory Bowel Disease</td>
<td>14,377</td>
<td>13,713</td>
</tr>
<tr>
<td>Parkinson Disease</td>
<td>27,200</td>
<td>23,939</td>
</tr>
</tbody>
</table>

Because of differences in which SNPs were genotyped and imputed between the amphetamine response dataset [Genetics of Amphetamine (GAP)] and the various datasets listed below, a slightly smaller number of SNPs was available for the enrichment analysis. IIBDGC, International Inflammatory Bowel Disease Genetics Consortium; PGC1, Psychiatric Genomics Consortium phase 1; PGC2, Psychiatric Genomics Consortium phase 2.