HHS Public Access

Author manuscript

Psychopharmacology (Berl). Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Psychopharmacology (Berl). 2015 August ; 232(15): 2781–2794. doi:10.1007/s00213-015-3914-1.

Multivariate Analysis of Subjective Responses to d-amphetamine In Healthy Volunteers Finds Novel Genetic Pathway Associations

Haley L. Yarosh, PhD[1,2], **Shashwath A. Meda, MS**[1], **Harriet de Wit, PhD**[5], **Amy B. Hart, B.Sc.**[4], and **Godfrey D. Pearlson, MD**[1,2,3]

[1]Olin Neuropsychiatry Research Center, Institute of Living at Hartford Hospital, Hartford, Connecticut

[2]Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut

[3]Department of Neurobiology, Yale University School of Medicine, New Haven, Connecticut

[4]Department of Human Genetics, University of Chicago, Chicago, Illinois

[5]Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, Illinois

Abstract

Rationale—Researchers studying behavioral and physiologic effects of d-amphetamine have explored individual response differences to the drug. Concurrently, genome wide analyses have identified several single nucleotide polymorphisms (SNPs) associated with these traits. Univariate methods can identify SNPs associated with behavioral and physiological traits, but multivariate analyses allow identification of clusters of related biologically relevant SNPs and behavioral components.

Objectives—To identify clusters of related biologically relevant SNPs and behavioral components in the responses of healthy individuals to d-amphetamine using multivariate analysis.

Methods—Individuals (N=375) without substance abuse histories completed surveys and detailed cardiovascular monitoring during randomized, blinded sessions: d-amphetamine (10mg, 20mg), placebo. We applied parallel-independent component analysis (Para-ICA) to data previously analyzed with univariate approaches, revealing new associations between genes and behavioral responses to d-amphetamine.

Results—Three significantly associated ($p<.001$) phenotype-genotype pairs emerged. The first component included physiologic measures of systolic and diastolic blood pressure (BP) and mean arterial pressure (MAP) along with SNPs in calcium and glutamatergic signaling pathways. The second associated components included the 'Anger' items from the Profile of Mood States (POMS) questionnaire and the Marijuana effects from the Addiction Research Center Inventory

Correspondence: Shashwath Meda, PhD Olin Neuropsychiatric Research Center 200 Retreat Avenue Hartford CT 06102 Phone: 860 545 7800 Fax: 860 545 7769 Shashwath.Meda@hhchealth.org.

Disclosure Statement

All authors have read and approved the manuscript, and none have any conflicts of interest. Dr. de Wit received a research grant from Unilever for a project unrelated to this study.

(Cuyas, Verdejo-Garcia et al.), with enriched genetic pathways involved in Cardiomyopathy and MAPK signaling. The final pair included 'Anxious', 'Fatigue', and 'Confusion' items from the POMS questionnaire, plus functional pathways related to cardiac muscle contraction and cardiomyopathy.

Conclusions—Multifactorial genetic networks related to calcium signaling, glutamatergic and dopaminergic synapse function and amphetamine addiction appear to mediate common behavioral and cardiovascular responses to d-amphetamine.

Keywords

D-amphetamine; Parallel-independent component analysis; Single nucleotide polymorphism; acute behavior

Introduction

Thus far, association studies of amphetamine response in healthy individuals have focused on candidate genes, employing univariate statistical approaches (Mattay, Goldberg et al. 2003, Wardle, Hart et al. 2013), which are typically limited by the issue of multiple comparisons, and diminish statistical power to detect subtle but important genetic effects. Thus, in the current study, we applied a novel, multivariate strategy based on parallel-independent component analysis (Para-ICA: see methods) to a previously analyzed data set.

Para-ICA is a multivariate statistical method that maximizes 'cost functions' both within and between complex feature sets (e.g. voxels in MRI scans and gene interactions). This reveals novel, biologically relevant associations that might otherwise not be detected due to small effect sizes and modest-sized sample sets (Liu, Pearlson et al. 2009, Meda, Narayanan et al. 2012). In data with a large number of characteristics, Para-ICA excels at identifying and comparing the most relevant features. Para-ICA also makes more assumptions about noise, thus increasing robustness compared to previous methods.

The current reanalysis used subjective and physiological response data to d-amphetamine in healthy individuals, and explored how these might be influenced by the genome of each participant. It is appropriate to apply Para-ICA to investigate relationships between complex behaviors and gene-gene interactions in this high dimensional data set.

D-amphetamine is a psychostimulant drug that acts acutely by blunting phasic dopamine release via agonist activity at D2 auto-receptors, and enhancing tonic availability of dopamine and norepinephrine by blocking reuptake via membrane transporters into nerve terminals (Greene, Kerr et al. 2008). Its effects include increased alertness/vigilance/arousal, energy, mood/motivation, attention/concentration and appetite suppression, and its side effects include hypertension, tachycardia, cardiac arrhythmias, cardiomyopathy, restlessness, dry mouth, and insomnia (Schep, Slaughter et al. 2010). Although amphetamine has therapeutic value for ADHD and treating fatigue, it also has the potential for abuse and dependence (Greene, Kerr et al. 2008).

There are individual differences in amphetamine response, and both the subjective response to acute amphetamine and amphetamine dependence are heritable (Crabbe, Jarvik et al.

1983, Schilt, Koeter et al. 2009, Cherner, Bousman et al. 2010). Some of the genetic variation is related to drug metabolism: for example methamphetamine users exhibit differential neurocognitive deficits related to specific *CYP2D6* drug metabolism-related alleles (Cherner, Bousman et al. 2010). Carriers of functional Catechol-O-methyltransferase (*COMT*) gene variants may show differential cognitive responses to acute *d*-amphetamine challenge (Mattay, Goldberg et al. 2003, Wardle, Hart et al. 2013), (Hamidovic, Dlugos et al. 2010). Although not directly related to drug challenges, individual differences in behavioral performance on executive and working memory tasks are also observed among polymorphism carriers of other genes relevant to dopaminergic function including *MAOA*, *BDNF* and *DRD4* (Fan, Fossella et al. 2003, Hariri and Weinberger 2003, Herrmann, Walter et al. 2007). To understand these individual differences, some of which may affect drug abuse susceptibility, it is important to investigate the genetic and physiological basis for subjective drug response in the general population (de Wit and Phillips 2012).

Our analysis was performed on a previously published sample that used univariate approaches, using varying sample sizes (*N*=99 or 162), to focus on carefully-selected, hypothesis-based candidate genes: *ADORA2A*, *SLC6A3*, *BDNF*, *SLC6A4*, *CSNK1E*, *SLC6A2*, *DRD2*, *FAAH*, *COMT*, and *OPRM1* (Hart, de Wit et al. 2013). Modest associations were reported, but these were difficult to replicate (Hart, de Wit et al. 2013). The authors also conducted a GWAS study in this population, with some modest findings (Hart, Engelhardt et al. 2012).

In the present analysis, we used an unsupervised multivariate approach to identify clusters of relevant interacting genes that were related in functional pathways to phenotypic components representing acute behavioral and physiologic response to d-amphetamine. Such genes could include those in processes previously associated with psychostimulant effects and/or dependence (Li, Mao et al. 2008), drug side effects (e.g. dry mouth, insomnia) as well as those related to drug metabolism and excretion, blood-brain barrier permeability, cardiovascular responsiveness, plus behaviorally-relevant neurotransmitters, receptors and their associated synthetic and degrading enzymes (e.g. dopamine, glutamate, norepinephrine). For the analysis, we generated change-from-pre-capsule baseline scores after placebo or drug to assess genotype-phenotype relationships. Several of the subjective scales had overlapping content, and some measures of somatic effects, e.g. heart rate, might influence subjective reports. Therefore, we expected some crossover between SNP components associated with behavioral and with physiological components.

The Para-ICA multivariate analysis used here provides a sensitive and powerful alternative to traditional univariate analyses using single SNPs and single outcome measures. It is more powerful than univariate analysis because it examines *clusters* of related individual phenotypic measures in relation to *clusters* of related SNPs. The clustering of phenotypic measures is empirically derived from the data, and the SNPs are interpreted based on a large body of knowledge about genes involved with known biological function. Para-ICA uses these two composite clusters to reveal novel, biologically relevant associations that might otherwise not be detected. Here, we applied this robust analysis to an existing data set with acute responses to amphetamine in human volunteers.

Materials and Methods

Participants

Study sample and data collection methods are detailed in previous publications (Hart, Engelhardt et al. 2012). Subjects were recruited and screened via modified Structured Clinical Interview for DSM-IV (SCID) (First November 2002) psychiatric symptom checklist (SCL-90) (Derogatis 1977) electrocardiogram, physical examination, self-reported health and drug use history. Subjects were excluded if they were employed with night shift work, significant medical or psychiatric conditions, if they smoked more than 3 cigarettes/day, consumed more than 3 cups of coffee/day, or tested positive for amphetamine, cocaine, opiates, phencyclidine (PCP), or marijuana (measured by urine toxicology: Ontrak TesTstik, Roche Diagnostic Systems Inc., Somerville, NJ).

Behavioral Data Collection

486 behavioral variables from the Drug Effects Questionnaire (DEQ; (Chait, Uhlenhuth et al. 1985), the Addiction Research Center Inventory (Martin, Sloan et al. 1971, Cuyas, Verdejo-Garcia et al.) and the Profile of Mood States (POMS; (Johanson and Uhlenhuth 1980) questionnaire were collected in addition to sex, age, education, body mass index, weekly alcohol and caffeine use as well as monthly marijuana use. These included baseline responses to placebo, 10mg d-amphetamine, and 20mg d-amphetamine as well as responses at five additional time points (30, 60, 90, 150 and 180 minutes post-drug). In order to understand changes in these variables compared to placebo, we grouped time points 2–6 into a single measure termed the ‘response measure’. We then created four change measures (CM): 1CM= 10mg baseline response - placebo baseline response 2CM= 20mg baseline response - placebo baseline response 3CM= 10mg response measure - placebo response measure 4CM= 20mg response measure - placebo response measure. Thus, 100 final behavioral variables were created for further analysis (Supplementary Figure I).

Genetic Data Collection

DNA was extracted from blood at the General Clinical Research Center at the University of Chicago. Genotyping was performed using the Affymetrix 6.0 array at the Functional Genomics Core Facility (Vanderbilt University, Memphis, TN, USA).

Genetic Data Processing

Prior to Para-ICA, genotyped SNPs underwent three pre-processing stages. First, quality control parameters were employed to discard data unsuitable for further analysis. Samples (both subjects and SNPs) were checked for missing data and those with missing call rates (>10%) were excluded. 375 individuals remained in the sample after excluding six individuals during quality control. Following this, all uninformative SNPs (constant variance) were excluded. SNPs were then checked for minor allele frequency (MAF); SNP variants with MAFs <0.1 were excluded. SNPs in linkage disequilibrium ($r^2 > 0.5$) (in block sizes of 100 kb) were removed. Finally, SNPs were checked for Hardy–Weinberg equilibrium set at a threshold of $p < 1\times10^{-5}$. The above analyses were performed using PLINK (Purcell, Neale et al. 2007). Finally, a principal component analysis (PCA) was run

using custom Matlab scripts and the variance from the top 3 components were regressed out of the data to account for population stratification using an approach similar to that implemented in EIGENSTRAT (Li and Yu 2008). All SNPs (N =178,746) were then carried over to the next processing stage.

In order to improve interpretation we biologically prioritized SNPs to only those contained within genes in KEGG database (Mootha, Lindgren et al. 2003, Kanehisa, Goto et al. 2004, Subramanian, Tamayo et al. 2005). In order to do this, we first derived genetic annotations for SNPs from the current dataset (conservatively) by using the Genome Variation Server (http://gvs.gs.washington.edu/GVSBatch137/) and restricting to SNPs within host genes (i.e. no flanking genes were used for SNP annotation). We then downloaded the complete list of KEGG genes from the molecular signatures database MSigDB (http://www.broadinstitute.org/gsea/msigdb/collections.jsp) and limited our analysis to only those that overlapped with the currently genotyped sample. This produced a biologically enriched SNP set of N=(13,751) that was used as input for the para-ICA analyses.

Parallel Independent Component Analysis

Association Mapping—The number of genetic components in the current study was estimated to be 6 and the number of components estimated for the behavioral networks was 13. Component estimation was data-driven using the standard minimum description length (MDL) criteria (Rissanen 1978).

To investigate associations between genotype and phenotype features, Para-ICA was implemented (See figure I) using the Fusion ICA Toolbox v2.0a; http://icatb.sourceforge.net in Matlab 7.0 and in accordance with previously reported results (Meda, Narayanan et al. 2012). Para-ICA was designed for multimodal processing that extracts components using an entropy term based on information theory to maximize independence, and enhances the interconnection by maximizing the linkage function in a joint estimation process (Calhoun, Liu et al. 2009). The goal of para-ICA was three-fold: a) run an ICA to extract distinct linear combinations of behavioral data (covarying phenotypic networks across subjects), b) simultaneously run another ICA to extract distinct, linear combinations of SNP data (covarying genetic networks across subjects) and c) maximize correlations between the derived networks from steps a and b. This process resulted in a number of components for each feature set that are variably expressed across subjects and quantified by a subject-level loading coefficient for each data type (See figure 1). The loading parameters from para-ICA represent the weight of the overall component for each subject (Calhoun, Adali et al. 2001, Schurz, Radua et al.). In para-ICA the correlation values between these loading parameters across the two feature sets are continuously updated and maximized, until a stopping criterion is reached. Comprehensive mathematical details for this methodology are also discussed in prior publications (Liu, Demirci et al. 2008).

In order to correct for multiple comparisons in resulting behavioral/genetic correlation values, Bonferroni correction was applied based on 13 (phenotype components)×6 (genetic components)=78 comparisons yielding a corrected p value threshold of 0.05/78 = 0.00064. Once significant phenotype-genotype associations were identified, the corresponding phenotype and genotype networks were thresholded at a supra level $|Z|>2.5$ to derive

significantly contributing elements from each feature set. SNPs/phenotypic variables surpassing this threshold were deemed to be contributing significantly to the overall signal of the corresponding component/network. Resulting significant genes were interpreted using a functional annotation tool, ConsensusPathDB-human (http://cpdb.molgen.mpg.de/) to visualize enriched biological networks that were associated with physiologic/behavioral responses identified within our multivariate framework. We selected KEGG from the pathway-based sets comparison option on this functional annotation tool.

Results

Overview

Participant demographics are detailed in previous publications (Hart, Engelhardt et al. 2012). Response to d-Amphetamine increased all scales of the DEQ, ARCI, and all but one of the POMS scales, and in a separate sparse factor analysis their responses to factors of “euphoria”, “dysphoria” and “arousal” were dose-dependent (Engelhardt and Stephens 2010).

The Para-ICA reanalysis identified three significant genotype-phenotype pairs after accounting for multiple comparisons using Bonferroni correction. The top 20 most significant genes from each of the genotype components G1, G2 and G3, their Z-scores and associated functional annotations are summarized in Table II. Enrichment of genes mapped to these individual SNPs yielded multiple significant pathways within the KEGG (Kanehisa and Goto 2000, Kanehisa, Goto et al. 2014) database. We report the top ten most significant pathways associated with each component in Table I.

Phenotypic behavioral responses are reported as change measures (CM), an average of all time points with baseline measures subtracted from this average. 1CM= 10mg baseline - placebo baseline 2CM= 20mg baseline - placebo baseline, 3CM= 10mg response measure - placebo response measure 4CM= 20mg response measure - placebo response measure.

Several functional pathways were enriched in all of our significant components, including dopaminergic (G1,G2,G3) and glutamatergic (G1,G2) signaling, consistent with the known mode of action of the drug. Interestingly, significant genes from our dataset (G1,G3) were also found to be over-represented in an ‘amphetamine addiction’ (not among top 10 pathways) KEGG pathway. As summarized earlier, multiple highly-ranked genes in all three SNP components have been linked to psychostimulant use/dependence, including *NRCAM* in G1 (Ishiguro, Liu et al. 2006) and *CREB5* in G2 (a modulator of *CART*), as well as *ACTB* in G3, *GABRG3* in G2 and *NCAM2* in G1.

Phenotype 1-Genotype 1 Association

The first phenotype component P1 derived by Para-ICA consisted of the physiologic measures of 3CM systolic blood pressure (BP), 3CM and 4CM measures of BP, and both 3CM and 4CM measures of mean arterial pressure (MAP). This component was negatively correlated to the first genetic component (G1; r=−.267, p=2.01e-007) (Table II) that included 211 significant SNPs, (thresholded at Z=2.5). Pathway enrichment analysis indicated that G1 consisted of calcium signaling networks ‘calcium signaling pathway’ and

'endocrine-regulated calcium reabsorption', plus a 'glutamatergic synapse' network. The calcium signaling pathways contain SNPs associated with Ca channel subunits and function (*CACNA1G, SLC8A3, SLC8A1*), G-protein coupled receptor and downstream pathway genes (*ADRA1A, PRKCB, ITPR1, ADCY2*), and nicotinic cholinergic receptor genes (*CHRNA7*). The glutamatergic pathway genes within this set were related to glutamate receptors (*GRIN2B, GRM1*) and phospholipase C (*PLCB1, PLCB2*). Other KEGG processes included vascular smooth-muscle contraction (that contained alpha-adrenergic-related genes including *ADRA1A)*, that are linked to amphetamine-mediated tachycardia and hypertension (Cruickshank and Dyer 2009), long-term depression, gap junction, circadian entrainment, salivary secretion (amphetamine causes dry mouth), amoebiasis (that contained genes relevant to the intestinal lining) and 'pathways in cancer'. Networks named 'dopaminergic synapse', 'cholinergic synapse', 'serotonergic synapse' and 'amphetamine addiction' (*GRIN2B, CREB5, PRKCB, CHP1, GNAS*) also contributed to the G1 component, but were not in the top ten most significant pathways.

Phenotype 2-Genotype 2 Association

Our second most significantly correlated pair (P2-G2; r=−.236, p=4.52e-006) included 4CM measures of the 'Anger' variables from the POMS questionnaire and 3CM measures from the Marijuana component of the ARCI (ARCI-M) and 229 significant SNPs (thresholded at z=2.5, see Table II) that were enriched in pathways related to somatic drug responses and drug metabolism (not in top 10 pathways). Importantly, three of eleven questions chosen for ARCI-M overlap with the amphetamine sub-score. Of the non-overlapping questions, many describe effects related to salivary secretion and/or cardiovascular function, e.g. "My mouth seems very dry," "I notice that my heart is beating faster." G2 pathways included 'dopaminergic synapse' (not among top 10 pathways), 'hippocampal signaling' (not among top 10 pathways), 'various forms of cardiomyopathy, (hypertrophic, arrhythmogenic right ventricular and dilated), 'adherens junction' and 'focal adhesion' (not in top 10 pathways), 'PI3K-Akt signaling', 'salivary' and 'pancreatic secretion' (not in top 10 pathways), 'MAP-kinase signaling'. Genes related to calcium channel regulation, vascular smooth muscle contraction and AMP-activated protein kinase were enriched in these pathways as well. Networks named 'morphine addiction' and 'nicotine addiction' also appeared to be significantly enriched but not among the top ten pathways for the G2 component. The top-ranked gene in this component, *CREB5*, is a *CART* interactor (Kuhar, Jaworski et al. 2005) that has been associated with substance abuse, as well as another top gene *GABRG3* (Table II). Amphetamine abuse has been linked to both acute and chronic cardiomyopathies (Greene, Kerr et al. 2008, Schep, Slaughter et al. 2010), and acute methamphetamine damages cardiomyocyte proteins (Turdi, Schamber et al. 2009) so the wide implication of related genes in G2 and G3 is unsurprising.

Phenotype 3-Genotype 3 Association

The third correlated pair P3-G3 (r=−.206, p=7.03e-005) was comprised of the 'Anxious', 'Fatigue', and 'Confusion' items from POMS questionnaire. It was significantly correlated with genotype component G3, which included 208 significant SNPs (thresholded at Z=2.5) and was enriched for several functional pathways related to cardiovascular function. This included some of the same networks discussed in G1 and G2 ('vascular smooth muscle

contraction', 'cardiac muscle contraction', 'arrhythmogenic right ventricular', 'dilated' and 'hypertrophic cardiomyopathies') (Table I). Additional enriched networks included 'focal adhesion', PI3k-Akt signaling', 'MAPK signaling pathway', 'calcium signaling pathway', and like G1 and G2, 'pathways in cancer'. The networks 'amphetamine addiction' and 'dopaminergic synapse' (discussed above) also appeared in G3 but were not among the top 10 pathways. The top SNP (*ACTB*) has been linked to methamphetamine-conditioned place preference (Shibasaki, Mizuno et al. 2011), and another top SNP (*PRKCE*) has been associated with substance use and cardiovascular stress (Nikpay, Seda et al. 2012).

We had hypothesized above that genes would be involved in functional pathways related to phenotypic components representing previously documented associations with acute behavioral and physiological responses to d-amphetamine. For the top- 20 ranked genes within each SNP network, this was true for 14 SNPs in component G1, and 16 in both G2 and G3, (represented e.g. as gene name-*Gx* for a particular gene comprising one of the top 20 in SNP component *Gx*) as follows:

Psychostimulant response: *NFATC3-G1*. Psychostimulant dependence/addiction vulnerability: *NRCAM-G1* (Ishiguro, Liu et al. 2006, Richardson, Grkovic et al. 2006), *CREB5-G2*, *GABRG3-G2*, *ACTB-G3*, *NCOR2-G3*, *PRKCE-G3*. Drug metabolism and excretion: *DPYD-G1*. Blood-brain barrier permeability: *CD44-G1*. Cardiovascular responsiveness: *NFATC3-G1*, *AP2A2-G1*, *SPTLC2-G2*, *RAB5A-G2*, *DHRS3-G2*, *SLC8A1-G2*, *CACNA2D3-G2*, *ST6GALNAC3-G3*. Vascular contractility/endothelium: *PLCB2-G1*, *ITGA8-G2*, *COLEC12-G2*, *PRKCQ-G3*, *KCNMA1-G3*, *PIK3C2B-G3*, *PRKCE-G3*. Behavior-relevant neurotransmitters- dopamine: *PARK2-G1*, *RORA-G1*, *RORB-G3*. Glutamate: *NCAM2-G1*, *GABA: GABRG3-G2*. Drug side effects - insomnia; *RORA-G1*, *RORB-G3*. General abused substance relevance e.g. cell adhesion processes (Ishiguro, Liu et al. 2006, Uhl, Drgon et al. 2008), *NCAM2-G1*, *COL4A2-G2*, *ITGA6-G2*. General brain development and/or neurotransmission: *ST8SIA1-G1*, *ERBB4-G1*, *TXNDC5-G1*, *SPTLC2-G2*, *PAK7-G2*, *GMDS-G2*, *BAIAP2-G2*, *ACTB-G3*, *ORC3-G3*, *MAPT-G3*, *WASF3-G3*, *B3GALTL-G3*, *KCNK10-G3*, *XYLT1-G3*, *DAPK1-G3*.

Discussion

The current analysis used Para-ICA, a novel multivariate approach, to identify pairs of related genotype and phenotype characteristics derived from subjective and physiological responses to two d-amphetamine doses compared to placebo in healthy individuals. We demonstrate that this empirically derived approach is a useful method for examining complex behavioral and cardiovascular responses to drugs where multiple, presumably interacting genes are involved. More traditional approaches (such as GWAS) produce findings that have been hard to replicate (Hart, de Wit et al. 2013), and require very large samples to achieve statistical significance.

Our results suggest that commonalities in d-amphetamine response may in part be explained by gene polymorphisms in several previously identified drug addiction-relevant pathways, and gene clusters related to cardiovascular function. The most significant association pair was G1-P1. Gene network G1 was negatively correlated with P1, indicating that decreased

3CM systolic BP, 3CM and 4CM measures of diastolic BP, and 3CM and 4CM MAP measures of mean arterial pressure (MAP) associates with increased G1 loading scores. Similarly, G2-P2 and G3-P3 were negatively correlated.

Although P1 subsumed only cardiovascular responsiveness measures, whereas phenotypes 2 and 3 contained exclusively behavioral self-report items, several of these latter subjective reports contained cardiovascular responsiveness items, (such as: “I noticed that my heart is beating faster”). In addition, many genes (such as those involved with many calcium and potassium channels, responsible for generating and conducting electrical impulses) are expressed both in brain and heart (Lu, McKinsey et al. 1999). This likely explains the mixture of SNPs belonging to genes identified with both cardiovascular and behavioral responses in all three gene components.

P1 comprised physiological drug response (HR, BP and MAP) and the corresponding G1 contained pathways for ‘long-term depression’, ‘circadian entrainment’, calcium and glutamatergic signaling. G1’s component network contains an enriched pathway for the glutamatergic synapse, including genes coding for the ionotropic glutamate receptor (*GRIN2B*), and *SLC1A1* encoding the excitatory aminoacid transporter 2 (*EAAT3*), required for removing glutamate from extracellular spaces. *GRIN2B* has been previously identified in inter-individual responses to the amphetamine MDMA via cognitive performance (Cuyas, Verdejo-Garcia et al. 2011).

Metabotropic glutamate receptor (mGLUR) genes (*GRM1*) are also represented in G1’s ‘calcium signaling’, and ‘glutamatergic synapse’ pathways, consistent with glutamate receptor involvement in cognitive control and amphetamine processing. Both acute DA and glutamate effects in rat brain, and the alteration of long-term potentiation via glutamatergic tone and metabotropic glutamate receptors are crucial to acute responses to psychostimulant drugs and addiction (Zhang, Loonam et al. 2001), (Kalivas 2009). The ventral medial prefrontal cortex, with abundant glutamatergic transmission, is involved in modulating bradycardic/tachycardic reflexes (Ferreira-Junior, Fedoce et al. 2013). Cardiac vagal preganglionic neurons are also responsible for heart rate modulation, and receive glutamatergic signaling input (Hildreth and Goodchild 2010).

Of the top 20 genes in the G1 component (Table II), several have been previously linked strongly to neuronal development and addiction vulnerability (*NRCAM* (Ishiguro, Liu et al. 2006), *NCAM2* (McIntyre, Titlow et al. 2010), *PRKG1* (Ishiguro, Liu et al. 2006, Uhl, Drgon et al. 2008)) or code for proteins affecting amphetamine metabolism (*DPYD*, *PARK2*, *RORA*, *NFATC3*, *GRID2*). For example, *NRCAM* knockout mice do not develop conditioned place preference for substances (amphetamine, cocaine, morphine,) that wild type mice do (Ishiguro, Liu et al. 2006).

Dopamine 1 receptor (D1R) agonists enhance stimulatory responses, and activate adenylyl cyclases, consistent with the high ranking of an adenylyl cyclase (*ADCY2*) gene in our G1 and G2 components; the top gene for G2 (*CREB5*) codes for cAMP-responsive element binding.

The ADCY family has been implicated in neural function and drug response (Procopio, Saba et al. 2013) as well as cardiac contractile response (Lipskaia, Defer et al. 2000). *ADCY2* interacts downstream with phospholipase C (PLC) signaling cascades; represented in the enriched pathways of our G1 component (*PLCB1*, *PLCB2*). PLC hydrolyses phosphatidylinositol 4,5-biphosphate (PIP_2) to create inositol 1,4,5-triphosphate receptor (IP3). The gene coding for IP3 receptor type 1 (*ITPR1*) is enriched in pathways of the G1 and G2 component.

A recent GWAS report of comorbid depression and alcoholism subjects (from the Consortium on the Genetics of Alcoholism, COGA) reported clusters of potential risk-conferring alleles similar to those described here (Edwards, Aliev et al. 2012) including glutamatergic genes (*GRIN2C* (Edwards, Aliev et al. 2012), *GRIN2B* (G1,G2), *GRIN2A* ((Edwards, Aliev et al. 2012), G3), *GRIA1* (Edwards, Aliev et al.), *GRIA4* (Edwards, Aliev et al.), and *GRM1* (G1,G2). *CTNNA2* is enriched in G2 and G3, while *CTNNA3* is enriched in pathways from our G2 component. *CTNNA2* has been identified in a large study of risk-taking propensity and excitement-seeking (Terracciano, Esko et al. 2011). Thus, there may be commonalities and functional gene networks underlying multiple types of substance abuse.

Enriched pathways in the G2 component include ‘ MAPK signaling’ ‘PI3K/Akt signaling’, ‘salivary secretion’, ‘hypertrophic cardiomyopathy’, ‘dilated cardiomyopathy’, ‘arrhythmogenic right ventricular cardiomyopathy’, and ‘circadian entrainment’.(Table I). Protein expression related to cardiovascular function would logically affect both subjective drug effects and physiological responses, as they involve generating, amplifying and transmitting cellular electrical signals in both heart and CNS. Heart rate increase in response to drug stimulant properties has been reported as a risk factor for substance abuse (Conrod, Peterson et al. 2001). Li and colleagues (Li, Mao et al. 2008) surveyed 1500 human addiction-related genes to identify molecular pathways significantly enriched for all four major classes of addictive drugs (Li, Mao et al. 2008). The authors then narrowed these genes to those supported by two or more studies, and finally to gene pathways enriched by these genes as compared to the whole genome. Among the most significant pathways identified were the MAPK (G2, G3) and calcium signaling (G1, G2, and G3) pathways along with the gap junction (G1). MAPK is also cited in relation to amphetamine exposure by Wang 2011 (Wang, Yuan et al. 2011) and Akt by Chen 2009 (Chen, Chen et al. 2009).

Salivary secretion decreases with amphetamine (Gotrick, Giglio et al. 2009), leading to complaints of dry mouth, and in part contributing to dental damage in chronic amphetamine abuse. *DHRS3* is a top gene in the G2 component, and is related to myocardial formation. The deletion or mutation of other top G2 genes result in cardiac failure, arrhythmia (*SLC8A1*) and stroke (*PRKCH*, *COL4A2*).

Another top-ranked gene appearing in G2 codes for the GABA-receptor subunit, *GABRG3*, identified as conferring genetic risk for alcoholism/alcohol withdrawal symptoms in COGA (Edenberg and Foroud 2006). This gene also appeared within our functional networks named ‘morphine addiction’ and ‘nicotine addiction’.

The G3 component includes enriched pathways previously discussed in G1 and G2 in addition to pathways named ‘focal adhesion’, ‘vascular smooth muscle contraction’, and ‘cardiac muscle contraction’. Adhesion molecules likely play an important role in methamphetamine risk (Uhl, Drgon et al. 2008).

This component contained multiple genes associated with neuronal development and with vascular smooth muscle contraction. Two among the top four, *ACTB* (Shibasaki, Mizuno et al. 2011) and *NCOR2*, are known to be associated with amphetamine and cocaine dependence, respectively; the second emerged as a gene important in cocaine dependence risk study (Gelernter, Sherva et al. 2013). Several cardiac genes in the G3 component are involved in smooth muscle function through calcium and potassium channel-related pathways (*KCNK10, KCNMA1, PIK3C2B, PRKCE, ST6GALNAC3*). *PRKCE* has also been implicated as a risk gene for substance use, and hyperactive stress response. Animals with *PRKCE* dysfunction show increased sensitive to alcohol and consume less alcohol than wild-type mice (Choi, Wang et al. 2002)

Genes in the CACNA family (*CACNG3, CACNA2D4, CACNA1A, CACNA1E, CACNA1G, CACNA2D3, CACNA2D1, CACNB2*) were represented in the ‘cardiac muscle contraction’ pathway and highly correlated with behavioral components in our G3 component. *CACNA1C* and *CACNA2D4* deletion have been implicated in bipolar disorder and schizophrenia susceptibility (Roussos, Bitsios et al. 2013). Similarly, a nicotinic cholinergic receptor gene enriched in pathways of G1 (*CHRNA7*) has been associated with schizophrenia (Freedman 2013). Amphetamine use is associated with symptomatic schizophrenia-like illnesses, likely associated with excessive dopamine release (Bramness, Gundersen et al. 2012).

In general, Para-ICA identified gene components related to drug use and abuse such as dopaminergic and glutamatergic signaling. Our results indicate a possible multi-loci genetic component encompassing individual genes playing crucial roles in drug response that may be markers for substance abuse susceptibility. Although we were unable to replicate the majority of previously reported genetic findings from the same subjects in univariate analyses (Hamidovic, Dlugos et al. 2010, Wardle, Hart et al. 2013); these initially-published findings were also not replicated in an expanded subject sample (Hart, de Wit et al. 2013).

Limitations

We recognize that our results are unreplicated, and that our analysis does not cover all genes or all neural genes. Our study is limited to functional annotation software categories and gene ontologies that are works-in-progress (Khatri, Sirota et al. 2012), and can contain functional annotation data based on prediction rather than experimental evidence. In addition, each of the pathway databases could have a inherent bias associated with them with respect to genes available within them given the nature of their initial field of inception. While our genetic pathways reflect the most current knowledge to date, the particular nomenclature may be transient. Our analysis also depends on SNPs with allele frequencies greater than 0.1 and restricting to KEGG related genes. The SNPs genotyped on the arrays may not capture some functional variants that are not in strong linkage disequilibrium.

Para-ICA should be considered for future analyses of behavioral-genetic relationships in individual drug responses. This multivariate analysis strategy compares relationships within and between two composite clusters, and identified several plausible network associations between d-amphetamine response and genetic variation. Both phenotypic and genotypic findings were generally predictable from known clinical acute behavioral and cardiovascular amphetamine effects and corresponding genes associated with related physiological brain and cardiovascular processes respectively, as well as genes associated with longer-term psychostimulant abuse/dependence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by NIH Grants DA007255 (ABH), T32AA015496-08 (HLY) and DA002812 (HdW).

References

Alibakhshi R, Moradi K, Mohebbi Z, Ghadiri K. Mutation analysis of PAH gene in patients with PKU in western Iran and its association with polymorphisms: identification of four novel mutations. Metab Brain Dis. 2013

Billings SE, Pierzchalski K, Butler Tjaden NE, Pang XY, Trainor PA, Kane MA, Moise AR. The retinaldehyde reductase DHRS3 is essential for preventing the formation of excess retinoic acid during embryonic development. FASEB J. 2013; 27(12):4877–4889. [PubMed: 24005908]

Bramness JG, Gundersen OH, Guterstam J, Rognli EB, Konstenius M, Loberg EM, Medhus S, Tanum L, Franck J. Amphetamine-induced psychosis--a separate diagnostic entity or primary psychosis triggered in the vulnerable? BMC Psychiatry. 2012; 12:221. [PubMed: 23216941]

Calhoun VD, Adali T, Pearlson GD, Pekar JJ. A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp. 2001; 14(3):140–151. [PubMed: 11559959]

Calhoun VD, Liu J, Adali T. A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data. Neuroimage. 2009; 45(1 Suppl):S163–172. [PubMed: 19059344]

Chait LD, Uhlenhuth EH, Johanson CE. The discriminative stimulus and subjective effects of d-amphetamine in humans. Psychopharmacology (Berl). 1985; 86(3):307–312. [PubMed: 3929301]

Chen JC, Chen PC, Chiang YC. Molecular mechanisms of psychostimulant addiction. Chang Gung Med J. 2009; 32(2):148–154. [PubMed: 19403004]

Cherner M, Bousman C, Everall I, Barron D, Letendre S, Vaida F, Atkinson JH, Heaton R, Grant I, Group H. Cytochrome P450-2D6 extensive metabolizers are more vulnerable to methamphetamine-associated neurocognitive impairment: preliminary findings. J Int Neuropsychol Soc. 2010; 16(5): 890–901. [PubMed: 20727252]

Choi DS, Wang D, Dadgar J, Chang WS, Messing RO. Conditional rescue of protein kinase C epsilon regulates ethanol preference and hypnotic sensitivity in adult mice. J Neurosci. 2002; 22(22):9905–9911. [PubMed: 12427847]

Conrod PJ, Peterson JB, Pihl RO. Reliability and validity of alcohol-induced heart rate increase as a measure of sensitivity to the stimulant properties of alcohol. Psychopharmacology (Berl). 2001; 157(1):20–30. [PubMed: 11512039]

Crabbe JC, Jarvik LF, Liston EH, Jenden DJ. Behavioral responses to amphetamines in identical twins. Acta Genet Med Gemellol (Roma). 1983; 32(2):139–149. [PubMed: 6685963]

Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. Addiction. 2009; 104(7):1085–1099. [PubMed: 19426289]

Cuyas E, Verdejo-Garcia A, Fagundo AB, Khymenets O, Rodriguez J, Cuenca A, de Sola Llopis S, Langohr K, Pena-Casanova J, Torrens M, Martin-Santos R, Farre M, de la Torre R. The influence of genetic and environmental factors among MDMA users in cognitive performance. PLoS One. 2011; 6(11):e27206. [PubMed: 22110616]

de Wit H, Phillips TJ. Do initial responses to drugs predict future use or abuse? Neurosci Biobehav Rev. 2012; 36(6):1565–1576. [PubMed: 22542906]

Deng PY, Xiao Z, Yang C, Rojanathammanee L, Grisanti L, Watt J, Geiger JD, Liu R, Porter JE, Lei S. GABA(B) receptor activation inhibits neuronal excitability and spatial learning in the entorhinal cortex by activating TREK-2 K+ channels. Neuron. 2009; 63(2):230–243. [PubMed: 19640481]

Derogatis, LR. SCL-90-R (Revised) Version Manual: I. Johns Hopkins University School of Medicine; 1977.

Di Battista ME, Pascale E, Purcaro C, Passarelli F, Passarelli E, Guglielmi R, Vanacore N, Meco G. Clinical subtypes in Parkinson's disease: the impact of MAPT haplotypes. J Neural Transm. 2013

Dick DM, Edenberg HJ, Xuei X, Goate A, Kuperman S, Schuckit M, Crowe R, Smith TL, Porjesz B, Begleiter H, Foroud T. Association of GABRG3 with alcohol dependence. Alcohol Clin Exp Res. 2004; 28(1):4–9. [PubMed: 14745296]

Edenberg HJ, Foroud T. The genetics of alcoholism: identifying specific genes through family studies. Addict Biol. 2006; 11(3–4):386–396. [PubMed: 16961766]

Edwards AC, Aliev F, Bierut LJ, Bucholz KK, Edenberg H, Hesselbrock V, Kramer J, Kuperman S, Nurnberger JI Jr, Schuckit MA, Porjesz B, Dick DM. Genome-wide association study of comorbid depressive syndrome and alcohol dependence. Psychiatr Genet. 2012; 22(1):31–41. [PubMed: 22064162]

Eker HK, Derinkuyu BE, Unal S, Masliah-Planchon J, Drunat S, Verloes A. Cerebro-fronto-facial syndrome type 3 with polymicrogyria: A clinical presentation of Baraitser-Winter syndrome. Eur J Med Genet. 2013

Elhayek D, Perez de Nanclares G, Chouchane S, Hamami S, Mlika A, Troudi M, Leban N, Ben Romdane W, Gueddiche MN, El Amri F, Mrabet S, Ben Chibani J, Castano L, Haj Khelil A, Ariceta G. Molecular diagnosis of distal renal tubular acidosis in Tunisian patients: proposed algorithm for Northern Africa populations for the ATP6V1B1, ATP6V0A4 and SCL4A1 genes. BMC Med Genet. 2013; 14(1):119. [PubMed: 24252324]

Engelhardt BE, Stephens M. Analysis of population structure: a unifying framework and novel methods based on sparse factor analysis. PLoS Genet. 2010; 6(9):e1001117. [PubMed: 20862358]

Eun LY, Song BW, Cha MJ, Song H, Kim IK, Choi E, Chang W, Lim S, Choi EJ, Ham O, Lee SY, Byun KH, Jang Y, Hwang KC. Overexpression of phosphoinositide-3-kinase class II alpha enhances mesenchymal stem cell survival in infarcted myocardium. Biochem Biophys Res Commun. 2010; 402(2):272–279. [PubMed: 20937252]

Fan J, Fossella J, Sommer T, Wu Y, Posner MI. Mapping the genetic variation of executive attention onto brain activity. Proc Natl Acad Sci U S A. 2003; 100(12):7406–7411. [PubMed: 12773616]

Ferreira-Junior NC, Fedoce AG, Alves FH, Resstel LB. Medial prefrontal cortex N-methyl-D-aspartate receptor/nitric oxide/cyclic guanosine monophosphate pathway modulates both tachycardic and bradycardic baroreflex responses. J Neurosci Res. 2013; 91(10):1338–1348. [PubMed: 23913674]

First, MBS.; RL; Miriam, Gibbon; Williams Janet, BW. Biometrics Research. New York State Psychiatric Institute; New York: Nov. 2002 Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. (SCID-I/NP).

Flynn KM, Michaud M, Madri JA. CD44 deficiency contributes to enhanced experimental autoimmune encephalomyelitis: a role in immune cells and vascular cells of the blood-brain barrier. Am J Pathol. 2013; 182(4):1322–1336. [PubMed: 23416161]

Freedman R. alpha7-Nicotinic Acetylcholine Receptor Agonists for Cognitive Enhancement in Schizophrenia. Annu Rev Med. 2013

Gelernter J, Sherva R, Koesterer R, Almasy L, Zhao H, Kranzler HR, Farrer L. Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. Mol Psychiatry. 2013

Gotrick B, Giglio D, Tobin G. Effects of amphetamine on salivary secretion. Eur J Oral Sci. 2009; 117(3):218–223. [PubMed: 19583747]

Greene SL, Kerr F, Braitberg G. Review article: amphetamines and related drugs of abuse. Emerg Med Australas. 2008; 20(5):391–402. [PubMed: 18973636]

Hainsworth AH, Allsopp RC, Jim A, Potter JF, Lowe J, Talbot CJ, Prettyman RJ. Death-associated protein kinase (DAPK1) in cerebral cortex of late-onset Alzheimer's disease patients and aged controls. Neuropathol Appl Neurobiol. 2010; 36(1):17–24. [PubMed: 19627511]

Hamidovic A, Dlugos A, Palmer AA, de Wit H. Catechol-O-methyltransferase val158met genotype modulates sustained attention in both the drug-free state and in response to amphetamine. Psychiatr Genet. 2010; 20(3):85–92. [PubMed: 20414144]

Hariri AR, Weinberger DR. Imaging genomics. Br Med Bull. 2003; 65:259–270. [PubMed: 12697630]

Hart AB, de Wit H, Palmer AA. Candidate gene studies of a promising intermediate phenotype: failure to replicate. Neuropsychopharmacology. 2013; 38(5):802–816. [PubMed: 23303064]

Hart AB, Engelhardt BE, Wardle MC, Sokoloff G, Stephens M, de Wit H, Palmer AA. Genome-wide association study of d-amphetamine response in healthy volunteers identifies putative associations, including cadherin 13 (CDH13). PLoS One. 2012; 7(8):e42646. [PubMed: 22952603]

Herrmann MJ, Walter A, Schreppel T, Ehlis AC, Pauli P, Lesch KP, Fallgatter AJ. D4 receptor gene variation modulates activation of prefrontal cortex during working memory. Eur J Neurosci. 2007; 26(10):2713–2718. [PubMed: 17970718]

Hildreth CM, Goodchild AK. Role of ionotropic GABA, glutamate and glycine receptors in the tonic and reflex control of cardiac vagal outflow in the rat. BMC Neurosci. 2010; 11:128. [PubMed: 20939929]

Hodgkinson CA, Enoch MA, Srivastava V, Cummins-Oman JS, Ferrier C, Iarikova P, Sankararaman S, Yamini G, Yuan Q, Zhou Z, Albaugh B, White KV, Shen PH, Goldman D. Genome-wide association identifies candidate genes that influence the human electroencephalogram. Proc Natl Acad Sci U S A. 2010; 107(19):8695–8700. [PubMed: 20421487]

Honjo Y, Ito H, Horibe T, Shimada H, Nakanishi A, Mori H, Takahashi R, Kawakami K. Derlin-1-immunopositive inclusions in patients with Alzheimer's disease. Neuroreport. 2012; 23(10):611–615. [PubMed: 22627700]

Ishiguro H, Hall FS, Horiuchi Y, Sakurai T, Hishimoto A, Grumet M, Uhl GR, Onaivi ES, Arinami T. NrCAM-regulating neural systems and addiction-related behaviors. Addict Biol. 2012

Ishiguro H, Liu QR, Gong JP, Hall FS, Ujike H, Morales M, Sakurai T, Grumet M, Uhl GR. NrCAM in addiction vulnerability: positional cloning, drug-regulation, haplotype-specific expression, and altered drug reward in knockout mice. Neuropsychopharmacology. 2006; 31(3):572–584. [PubMed: 16123759]

Jayanthi S, Deng X, Ladenheim B, McCoy MT, Cluster A, Cai NS, Cadet JL. Calcineurin/NFAT-induced up-regulation of the Fas ligand/Fas death pathway is involved in methamphetamine-induced neuronal apoptosis. Proc Natl Acad Sci U S A. 2005; 102(3):868–873. [PubMed: 15644446]

Johanson CE, Uhlenhuth EH. Drug preference and mood in humans: d-amphetamine. Psychopharmacology (Berl). 1980; 71(3):275–279. [PubMed: 6779335]

Kalivas PW. The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci. 2009; 10(8):561–572. [PubMed: 19571793]

Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000; 28(1):27–30. [PubMed: 10592173]

Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resource for deciphering the genome. Nucleic Acids Res. 2004; 32(Database issue):D277–280. [PubMed: 14681412]

Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res. 2014; 42(Database issue):D199–205. [PubMed: 24214961]

Khananshvili D. The SLC8 gene family of sodium-calcium exchangers (NCX) - structure, function, and regulation in health and disease. Mol Aspects Med. 2013; 34(2–3):220–235. [PubMed: 23506867]

Khatri P, Sirota M, Butte AJ. Ten years of pathway analysis: current approaches and outstanding challenges. PLoS Comput Biol. 2012; 8(2):e1002375. [PubMed: 22383865]

Kitazono T, Kubo M, Hata J, Ibayashi S, Kiyohara Y, Iida M. Molecular epidemiology of cerebrovascular diseases; the Hisayama study and the Fukuoka Stroke Registry (FSR). Rinsho Shinkeigaku. 2008; 48(11):892–895. [PubMed: 19198108]

Kuhar MJ, Jaworski JN, Hubert GW, Philpot KB, Dominguez G. Cocaine- and amphetamine-regulated transcript peptides play a role in drug abuse and are potential therapeutic targets. AAPS J. 2005; 7(1):E259–265. [PubMed: 16146347]

Kuo DS, Labelle-Dumais C, Gould DB. COL4A1 and COL4A2 mutations and disease: insights into pathogenic mechanisms and potential therapeutic targets. Hum Mol Genet. 2012; 21(R1):R97–110. [PubMed: 22914737]

LaBelle EF, Polyak F. Phospholipase C beta 2 in vascular smooth muscle. J Cell Physiol. 1996; 169(2):358–363. [PubMed: 8908203]

Lalonde R, Strazielle C. Discrimination learning in Rora(sg) and Grid2(ho) mutant mice. Neurobiol Learn Mem. 2008; 90(2):472–474. [PubMed: 18583162]

Li CY, Mao X, Wei L. Genes and (common) pathways underlying drug addiction. PLoS Comput Biol. 2008; 4(1):e2. [PubMed: 18179280]

Li Q, Yu K. Improved correction for population stratification in genome-wide association studies by identifying hidden population structures. Genet Epidemiol. 2008; 32(3):215–226. [PubMed: 18161052]

Lin SH, Liu CM, Liu YL, Shen-Jang Fann C, Hsiao PC, Wu JY, Hung SI, Chen CH, Wu HM, Jou YS, Liu SK, Hwang TJ, Hsieh MH, Chang CC, Yang WC, Lin JJ, Chou FH, Faraone SV, Tsuang MT, Hwu HG, Chen WJ. Clustering by neurocognition for fine mapping of the schizophrenia susceptibility loci on chromosome 6p. Genes Brain Behav. 2009; 8(8):785–794. [PubMed: 19694819]

Lipskaia L, Defer N, Esposito G, Hajar I, Garel MC, Rockman HA, Hanoune J. Enhanced cardiac function in transgenic mice expressing a Ca(2+)-stimulated adenylyl cyclase. Circ Res. 2000; 86(7):795–801. [PubMed: 10764414]

Liu J, Demirci O, Calhoun VD. A Parallel Independent Component Analysis Approach to Investigate Genomic Influence on Brain Function. IEEE Signal Process Lett. 2008; 15:413–416. [PubMed: 19834575]

Liu J, Pearlson G, Windemuth A, Ruano G, Perrone-Bizzozero NI, Calhoun V. Combining fMRI and SNP data to investigate connections between brain function and genetics using parallel ICA. Hum Brain Mapp. 2009; 30(1):241–255. [PubMed: 18072279]

Logue MW, Baldwin C, Guffanti G, Melista E, Wolf EJ, Reardon AF, Uddin M, Wildman D, Galea S, Koenen KC, Miller MW. A genome-wide association study of post-traumatic stress disorder identifies the retinoid-related orphan receptor alpha (RORA) gene as a significant risk locus. Mol Psychiatry. 2013; 18(8):937–942. [PubMed: 22869035]

Lu JR, McKinsey TA, Xu H, Wang DZ, Richardson JA, Olson EN. FOG-2, a heart- and brain-enriched cofactor for GATA transcription factors. Mol Cell Biol. 1999; 19(6):4495–4502. [PubMed: 10330188]

Martin WR, Sloan JW, Sapira JD, Jasinski DR. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin Pharmacol Ther. 1971; 12(2):245–258. [PubMed: 5554941]

Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, Kolachana B, Callicott JH, Weinberger DR. Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc Natl Acad Sci U S A. 2003; 100(10):6186–6191. [PubMed: 12716966]

McGrath CL, Glatt SJ, Sklar P, Le-Niculescu H, Kuczenski R, Doyle AE, Biederman J, Mick E, Faraone SV, Niculescu AB, Tsuang MT. Evidence for genetic association of RORB with bipolar disorder. BMC Psychiatry. 2009; 9:70. [PubMed: 19909500]

McIntyre JC, Titlow WB, McClintock TS. Axon growth and guidance genes identify nascent, immature, and mature olfactory sensory neurons. J Neurosci Res. 2010; 88(15):3243–3256. [PubMed: 20882566]

Meda SA, Narayanan B, Liu J, Perrone-Bizzozero NI, Stevens MC, Calhoun VD, Glahn DC, Shen L, Risacher SL, Saykin AJ, Pearlson GD. A large scale multivariate parallel ICA method reveals novel imaging-genetic relationships for Alzheimer's disease in the ADNI cohort. Neuroimage. 2012; 60(3):1608–1621. [PubMed: 22245343]

Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet. 2003; 34(3):267–273. [PubMed: 12808457]

Murphy SM, Ernst D, Wei Y, Laura M, Liu YT, Polke J, Blake J, Winer J, Houlden H, Hornemann T, Reilly MM. Hereditary sensory and autonomic neuropathy type 1 (HSANI) caused by a novel mutation in SPTLC2. Neurology. 2013; 80(23):2106–2111. [PubMed: 23658386]

Nekrasova T, Jobes ML, Ting JH, Wagner GC, Minden A. Targeted disruption of the Pak5 and Pak6 genes in mice leads to deficits in learning and locomotion. Dev Biol. 2008; 322(1):95–108. [PubMed: 18675265]

Ng MC, Tam CH, So WY, Ho JS, Chan AW, Lee HM, Wang Y, Lam VK, Chan JC, Ma RC. Implication of genetic variants near NEGR1, SEC16B, TMEM18, ETV5/DGKG, GNPDA2, LIN7C/BDNF, MTCH2, BCDIN3D/FAIM2, SH2B1, FTO, MC4R, and KCTD15 with obesity and type 2 diabetes in 7705 Chinese. J Clin Endocrinol Metab. 2010; 95(5):2418–2425. [PubMed: 20215397]

Nikpay M, Seda O, Tremblay J, Petrovich M, Gaudet D, Kotchen TA, Cowley AW Jr, Hamet P. Genetic mapping of habitual substance use, obesity-related traits, responses to mental and physical stress, and heart rate and blood pressure measurements reveals shared genes that are overrepresented in the neural synapse. Hypertens Res. 2012; 35(6):585–591. [PubMed: 22297481]

Orsetti M, Di Brisco F, Canonico PL, Genazzani AA, Ghi P. Gene regulation in the frontal cortex of rats exposed to the chronic mild stress paradigm, an animal model of human depression. Eur J Neurosci. 2008; 27(8):2156–2164. [PubMed: 18371075]

Procopio DO, Saba LM, Walter H, Lesch O, Skala K, Schlaff G, Vanderlinden L, Clapp P, Hoffman PL, Tabakoff B. Genetic markers of comorbid depression and alcoholism in women. Alcohol Clin Exp Res. 2013; 37(6):896–904. [PubMed: 23278386]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–575. [PubMed: 17701901]

Ribases M, Bosch R, Hervas A, Ramos-Quiroga JA, Sanchez-Mora C, Bielsa A, Gastaminza X, Guijarro-Domingo S, Nogueira M, Gomez-Barros N, Kreiker S, Gross-Lesch S, Jacob CP, Lesch KP, Reif A, Johansson S, Plessen KJ, Knappskog PM, Haavik J, Estivill X, Casas M, Bayes M, Cormand B. Case-control study of six genes asymmetrically expressed in the two cerebral hemispheres: association of BAIAP2 with attention-deficit/hyperactivity disorder. Biol Psychiatry. 2009; 66(10):926–934. [PubMed: 19733838]

Richardson RJ, Grkovic I, Anderson CR. Cocaine- and amphetamine-related transcript peptide and somatostatin in rat intracardiac ganglia. Cell Tissue Res. 2006; 324(1):17–24. [PubMed: 16374620]

Roussos P, Bitsios P, Giakoumaki SG, McClure MM, Hazlett EA, New AS, Siever LJ. CACNA1C as a risk factor for schizotypal personality disorder and schizotypy in healthy individuals. Psychiatry Res. 2013; 206(1):122–123. [PubMed: 22985546]

Sarachana T, Hu VW. Genome-wide identification of transcriptional targets of RORA reveals direct regulation of multiple genes associated with autism spectrum disorder. Mol Autism. 2013; 4(1):14. [PubMed: 23697635]

Schep LJ, Slaughter RJ, Beasley DM. The clinical toxicology of metamfetamine. Clin Toxicol (Phila). 2010; 48(7):675–694. [PubMed: 20849327]

Schilt T, Koeter MW, de Win MM, Zinkstok JR, van Amelsvoort TA, Schmand B, den Brink W. The effect of Ecstasy on memory is moderated by a functional polymorphism in the cathechol-O-methyltransferase (COMT) gene. Eur Neuropsychopharmacol. 2009; 19(2):116–124. [PubMed: 19042106]

Schoner K, Kohlhase J, Muller AM, Schramm T, Plassmann M, Schmitz R, Neesen J, Wieacker P, Rehder H. Hydrocephalus, agenesis of the corpus callosum, and cleft lip/palate represent frequent associations in fetuses with Peters' plus syndrome and B3GALTL mutations. Fetal PPS phenotypes, expanded by Dandy Walker cyst and encephalocele. Prenat Diagn. 2013; 33(1):75–80. [PubMed: 23161355]

Schurz M, Radua J, Aichhorn M, Richlan F, Perner J. Fractionating theory of mind: A meta-analysis of functional brain imaging studies. Neurosci Biobehav Rev. 2014

Shibasaki M, Mizuno K, Kurokawa K, Suzuki T, Ohkuma S. Role of actin depolymerizing factor in the development of methamphetamine-induced place preference in mice. Eur J Pharmacol. 2011; 671(1–3):70–78. [PubMed: 21978834]

Singleton AB, Farrer MJ, Bonifati V. The genetics of Parkinson's disease: progress and therapeutic implications. Mov Disord. 2013; 28(1):14–23. [PubMed: 23389780]

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005; 102(43):15545–15550. [PubMed: 16199517]

Supriyanto I, Watanabe Y, Mouri K, Shiroiwa K, Ratta-Apha W, Yoshida M, Tamiya G, Sasada T, Eguchi N, Okazaki K, Shirakawa O, Someya T, Hishimoto A. A missense mutation in the ITGA8 gene, a cell adhesion molecule gene, is associated with schizophrenia in Japanese female patients. Prog Neuropsychopharmacol Biol Psychiatry. 2013; 40:347–352. [PubMed: 23153507]

Terracciano A, Esko T, Sutin AR, de Moor MH, Meirelles O, Zhu G, Tanaka T, Giegling I, Nutile T, Realo A, Allik J, Hansell NK, Wright MJ, Montgomery GW, Willemsen G, Hottenga JJ, Friedl M, Ruggiero D, Sorice R, Sanna S, Cannas A, Raikkonen K, Widen E, Palotie A, Eriksson JG, Cucca F, Krueger RF, Lahti J, Luciano M, Smoller JW, van Duijn CM, Abecasis GR, Boomsma DI, Ciullo M, Costa PT Jr, Ferrucci L, Martin NG, Metspalu A, Rujescu D, Schlessinger D, Uda M. Meta-analysis of genome-wide association studies identifies common variants in CTNNA2 associated with excitement-seeking. Transl Psychiatry. 2011; 1:e49. [PubMed: 22833195]

Thomas EA, Foye PE, Alvarez CE, Usui H, Sutcliffe JG. Insulin receptor substrate protein p53 localization in rats suggests mechanism for specific polyglutamine neurodegeneration. Neurosci Lett. 2001; 309(3):145–148. [PubMed: 11514062]

Toma C, Hervas A, Balmana N, Vilella E, Aguilera F, Cusco I, del Campo M, Caballero R, De Diego-Otero Y, Ribases M, Cormand B, Bayes M. Association study of six candidate genes asymmetrically expressed in the two cerebral hemispheres suggests the involvement of BAIAP2 in autism. J Psychiatr Res. 2011; 45(2):280–282. [PubMed: 20888579]

Turdi S, Schamber RM, Roe ND, Chew HG Jr, Culver B, Ren J. Acute methamphetamine exposure inhibits cardiac contractile function. Toxicol Lett. 2009; 189(2):152–158. [PubMed: 19481142]

Uhl GR, Drgon T, Liu QR, Johnson C, Walther D, Komiyama T, Harano M, Sekine Y, Inada T, Ozaki N, Iyo M, Iwata N, Yamada M, Sora I, Chen CK, Liu HC, Ujike H, Lin SK. Genome-wide association for methamphetamine dependence: convergent results from 2 samples. Arch Gen Psychiatry. 2008; 65(3):345–355. [PubMed: 18316681]

Wang J, Yuan W, Li MD. Genes and pathways co-associated with the exposure to multiple drugs of abuse, including alcohol, amphetamine/methamphetamine, cocaine, marijuana, morphine, and/or nicotine: a review of proteomics analyses. Mol Neurobiol. 2011; 44(3):269–286. [PubMed: 21922273]

Wang L, Zheng Y, Xu H, Yan X, Chang X. Investigate pathogenic mechanism of TXNDC5 in rheumatoid arthritis. PLoS One. 2013; 8(1):e53301. [PubMed: 23326410]

Wardle MC, Hart AB, Palmer AA, de Wit H. Does COMT genotype influence the effects of d-amphetamine on executive functioning? Genes Brain Behav. 2013; 12(1):13–20. [PubMed: 23231539]

Wolf A, Agnihotri S, Munoz D, Guha A. Developmental profile and regulation of the glycolytic enzyme hexokinase 2 in normal brain and glioblastoma multiforme. Neurobiol Dis. 2011; 44(1): 84–91. [PubMed: 21726646]

Zhang Y, Loonam TM, Noailles PA, Angulo JA. Comparison of cocaine- and methamphetamine-evoked dopamine and glutamate overflow in somatodendritic and terminal field regions of the rat

brain during acute, chronic, and early withdrawal conditions. Ann N Y Acad Sci. 2001; 937:93–120. [PubMed: 11458542]

Zitman FM, Todorov B, Verschuuren JJ, Jacobs BC, Furukawa K, Furukawa K, Willison HJ, Plomp JJ. Neuromuscular synaptic transmission in aged ganglioside-deficient mice. Neurobiol Aging. 2011; 32(1):157–167. [PubMed: 19233512]

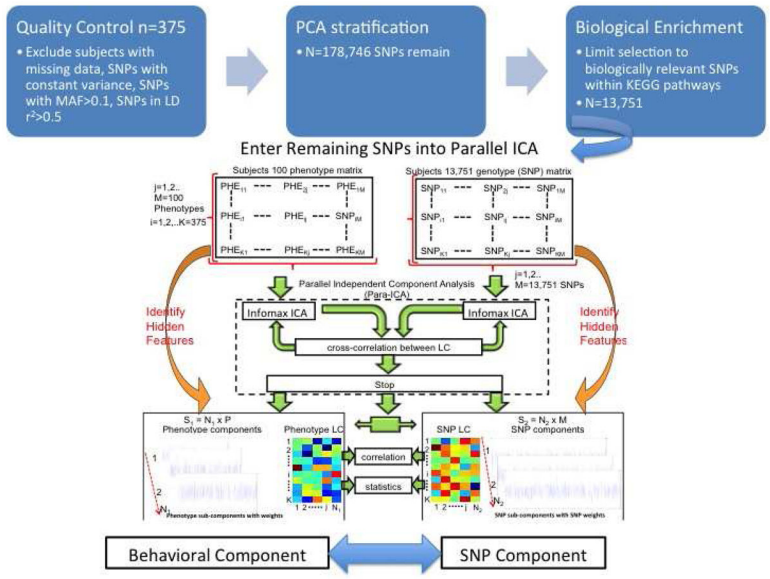

Figure 1.
Parallel Independent Component Analysis Study flow

Psychopharmacology (Berl). Author manuscript; available in PMC 2016 August 01.

Table I

List of Top 10 processes using KEGG database for G1, G2 & G3

G1	p-value	q-value	Genes implicated
Calcium signaling pathway	5.96E-13	9.66E-11	*ADRA1A; PLCB1; PLCB2; PRKCB; ERBB4; ADCY2; GNAS; RYR3; RYR2; CHP1; PTGFR; CACNA1G; ITPKA; SLC8A1; GNA14; CHRNA7; GNAL; SLC8A3; ITPR1; GRM1*
Long-term depression	4.44E-10	3.05E-08	*PLCB1; PLCB2; GRID2; PRKCB; GUCY1A2; PRKG1; GUCY1B3; PLA2G4A; ITPR1; GRM1; GNAS*
Circadian entrainment	5.65E-10	3.05E-08	*PLCB1; PLCB2; PRKCB; ADCY2; GNAS; RYR3; RYR2; CACNA1G; GRIN2B; GUCY1A2; PRKG1; GUCY1B3; ITPR1*
Salivary secretion	3.21E-09	1.30E-07	*ADRA1A; PLCB1; PLCB2; ADCY2; GNAS; RYR3; PRKCB; ATP1B1; GUCY1A2; PRKG1; GUCY1B3; ITPR1*
Vascular smooth muscle contraction	1.02E-08	3.32E-07	*ADRA1A; PLCB1; PLCB2; ADCY2; GNAS; PRKCB; PRKCE; GUCY1A2; PRKG1; PRKCH; GUCY1B3; PLA2G4A; ITPR1*
Endocrine and other factor- regulated calcium reabsorption	2.53E-07	6.47E-06	*PLCB1; PLCB2; GNAS; PRKCB; ATP1B1; DNM3; AP2A2; SLC8A1*
Amoebiasis	2.79E-07	6.47E-06	*LAMA1; PLCB2; PRKCB; PLCB1; GNAS; SERPINB1; GNA14; COL4A1; GNAL; LAMC3; LAMC1*
Gap junction	3.61E-07	7.31E-06	*PLCB1; PLCB2; ADCY2; GNAS; PRKCB; GUCY1A2; PRKG1; GUCY1B3; ITPR1; GRM1*
Pathways in cancer	5.26E-07	9.32E-06	*BCL2L1; LAMA1; TGFBR1; PRKCB; CDK6; RXRG; TCF7L1; RET; AKT2; ETS1; CREBBP; COL4A1; MITF; LAMC3; CTBP2; LAMC1; DAPK1; FGF14*
Glutamatergic synapse	5.75E-07	9.32E-06	*PLCB1; PLCB2; ADCY2; GNAS; PRKCB; CHP1; SLC1A1; GRIN2B; PLA2G4A; ITPR1; GRM1*
G2			
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3.95E-10	4.71E-08	*ITGA8; ACTN2; ITGB3; ITGA6; CACNB2; TCF7L2; CTNNA2; SLC8A1; CACNA2D3; CACNG3; CACNA2D4; CTNNA3*
Calcium signaling pathway	6.69E-10	4.71E-08	*LHCGR; ATP2B2; ADRA1D; ADCY2; EGFR; RYR3; PRKCB; CHRM3; CACNA1G; GRM1; CHRNA7; SLC8A1; AGTR1; ITPR1; GRIN2A; ATP2B4; GNAQ*
Hypertrophic cardiomyopathy (HCM)	1.98E-08	9.29E-07	*ITGA8; TGFB2; CACNB2; PRKAG2; PRKAA2; ITGA6; ITGB3; SLC8A1; CACNA2D3; CACNG3; CACNA2D4*
Salivary secretion	4.68E-08	1.65E-06	*ATP2B2; ADRA1D; ADCY2; ATP1B2; RYR3; PRKCB; CHRM3; PRKG1; ITPR1; ATP2B4; GNAQ*
MAPK signaling pathway	1.18E-07	3.32E-06	*TGFBR2; TGFB2; RASGRF1; CACNB2; PRKCB; EGFR; NTRK2; CACNA1G; FASLG; MAPT; CACNA2D3; FGF12; FLNB; CACNG3; FGFR2; IL1R2; CACNA2D4*
Pathways in cancer	1.48E-07	3.47E-06	*WNT2B; TGFBR2; TGFB2; RALBP1; STAT1; WNT2; BID; PRKCB; EGFR; DCC; ITGA6; FASLG; COL4A2; TCF7L2; FGF12; FGFR2; DAPK2; CTNNA2; CTNNA3*
Dilated cardiomyopathy	4.86E-07	9.79E-06	*ITGA8; ITGB3; ADCY2; CACNB2; TGFB2; ITGA6; SLC8A1; CACNA2D3; CACNG3; CACNA2D4*
PI3K-Akt signaling pathway	6.65E-06	0.00011	*ITGA8; ITGB3; TEK; EGFR; CREB5; PPP2R2A; PRKAA2; ITGA6; PPP2R2B; FASLG; COL4A2; TNC; FGF12; VWF; ANGPT1; FGFR2; PPP2R5A*
Circadian entrainment	7.70E-06	0.00011	*GNAQ; ADCY2; RYR3; PRKCB; CACNA1G; GRIN2B; GRIN2A; PRKG1; ITPR1*
Adherens junction	7.83E-06	0.00011	*TGFBR2; ACTN2; EGFR; TCF7L2; BAIAP2; PTPRM; CTNNA2; CTNNA3*

G1	p-value	q-value	Genes implicated
G3			
Dilated cardiomyopathy	7.17E-12	9.39E-10	*TGFB2; CACNA1S; CACNA2D3; GNAS; ITGA1; CACNA1C; CACNA1D; MYL2; MYBPC3; CACNB2; SLC8A1; PLN; ACTB; CACNG3*
Hypertrophic cardiomyopathy (HCM)	3.82E-11	2.50E-09	*TGFB2; CACNG3; CACNB2; CACNA1C; CACNA1D; PRKAG2; MYL2; MYBPC3; ITGA1; SLC8A1; CACNA2D3; ACTB; CACNA1S*
Calcium signaling pathway	1.74E-10	7.61E-09	*ADRA1A; CACNA1I; PTGFR; PRKCB; GNAS; RYR3; CACNA1C; CACNA1D; PTGER3; GNA14; ITPKB; OXTR; SLC8A1; PLN; CACNA1S; TACR1; GRIN2A*
MAPK signaling pathway	5.16E-09	1.69E-07	*CACNA1I; TGFB2; CACNA1S; RASGRF1; RASGRP3; MAP3K3; CACNA1C; CACNA1D; CHUK; PRKCB; NTF3; MAP2K6; MAPT; CACNB2; CACNA2D3; CACNG3; FGFR2; FGF14*
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3.30E-08	8.64E-07	*CACNA1S; CACNB2; CACNA1C; CACNA1D; ITGA1; SLC8A1; CACNA2D3; ACTB; CACNG3; CTNNA2*
Pathways in cancer	1.98E-07	4.33E-06	*LAMA1; TGFB2; MSH3; RALBP1; CTNNA2; PRKCB; CSF1R; CHUK; GLI2; GLI3; ETS1; COL4A2; COL4A1; WNT5B; CTBP2; FGFR2; DAPK1; FGF14*
PI3K-Akt signaling pathway	2.07E-06	3.87E-05	*LAMA1; IL6R; EIF4E2; PDGFD; SGK1; ITGA1; CSF1R; CHUK; PPP2R2B; RELN; COL4A2; COL4A1; COL6A1; COL6A3; ANGPT1; FGFR2; FGF14*
Focal adhesion	2.46E-06	4.03E-05	*LAMA1; PDGFD; RASGRF1; VAV1; ITGA1; PRKCB; MYL2; RELN; COL4A1; COL6A1; ACTB; COL6A3; COL4A2*
Vascular smooth muscle contraction	3.47E-06	5.06E-05	*ADRA1A; PRKCB; GNAS; PRKCE; CACNA1C; CACNA1D; KCNMA1; PRKG1; PRKCQ; CACNA1S*
Cardiac muscle contraction	5.68E-06	7.44E-05	*CACNA1S; CACNB2; CACNA1C; CACNA1D; MYL2; SLC8A1; CACNA2D3; CACNG3*

Table reports significant values from a standard enrichment analysis derived from the CPDB database. Top 10 enriched KEGG process IDs are noted along with the significant genes in the current study that are involved in these processes. Uncorrected p and corresponding FDR corrected q values are also reported in this table. Gene components G1,G2 and G3 were significantly associated with phenotypic components P1, P2 and P3 respectively.

Psychopharmacology (Berl). Author manuscript; available in PMC 2016 August 01.

Table 2

List of top 20 SNPs/genes and their function for each significantly correlated components

Gene	Chromosome/Position	SNP	Pathway/Function	Disease association	|Z| score
G1					
PARK2	6:161860169	rs9458252	DAT function	Parkinson's disease (Singleton, Farrer et al. 2013)	4.7709134
PLCB2	15:40590265	rs2305648	Vascular smooth muscle	Cardiovascular (LaBelle and Polyak 1996)	−4.1793759
CD44	11:35200916	rs3794105	Vascular/blood-brain barrier	Encephalomyelitis (Flynn, Michaud et al. 2013)	3.9902939
TXNDC5	6:7896491	rs443861	Protein folding	Schizophrenia, Arthritis (Lin, Liu et al. 2009, Wang, Zheng et al. 2013)	3.8772794
CD44	11:35184823	rs353635	See above	See above	3.8637463
NRCAM	7:107908197	rs1269659	Neural circuit formation	Addiction vulnerability (Ishiguro, Hall et al. 2012)	−3.7852901
RORA	15:61433543	rs11071587	DA/GLU signaling, circadian rhythms, learning (Lalonde and Strazielle 2008)	Autism (Sarachana and Hu 2013), PTSD (Logue, Baldwin et al. 2013)	3.7836012
NFATC3	16:68154862	rs2418736	Neural-based amphetamine response (Jayanthi, Deng et al. 2005) Cardiovascular muscle		−3.6746777
ACSM5	16:20439718	rs6497484			3.6730504
ST8SIA1	12:22473320	rs2541299	Calcium Signaling	Neuromuscular junction (Zitman, Todorov et al. 2011)	−3.6606826
AP2A2	11:942344	rs10751669	Cardiac muscle function	Cardiovascular disease	3.6062236
ERBB4	2:212376745	rs2030457	Neural/Cardiovascular development		−3.559317
NCAM2	21:22494449	rs2826663	Cell adhesion molecule	Addiction Vulnerability	3.5584064
GRID2	4:94312343	rs1948016	Glutamate Receptor	Neurodegenerative disorders	3.5515802
HPSE2	10:100224362	rs754586	ECM structure, angiogenesis	Urofacial syndrome	3.5447522
NUP153	6:17627590	rs4716167	DNA repair	HIV	−3.5299582
GALNTL6	4:172849152	rs10005702			−3.4903409

Gene	Chromosome/Position	SNP	Pathway/Function	Disease association	|Z| score
DPYD	1:97895762	rs11165870	Drug metabolism marker		3.4758147
PRODH	22:18917031	rs367766	Phosphatidylinositol signaling		−3.4520486
DGKG	3:185916113	rs2268846	Lipid metabolism	Obesity (Ng, Tam et al. 2010)	−3.4225716
ATP6V1H	8:54628736	rs3735833	ATPAse, Protein folding		−3.4225603
G2					
CREB5	7:28443938	rs42704	DA/Cholinergic synapses	Drug addiction	−4.6509652
TNC	9:117840922	rs1330351	Inflammation		−4.2077072
SPTLC2	14:78057835	rs2142187	Neural and cardiovascular function	Neuropathy (Murphy, Ernst et al. 2013)	−4.1203353
ITGA8	10:15638751	rs1057969	Smooth muscle contraction	Schizophrenia (Supriyanto, Watanabe et al. 2013)	−3.8815892
RAB5A	3:20022495	rs12488378	GTP-binding protein, cardiovascular function		−3.8546808
COL4A2	7:28432434	rs387344	PI3K-Akt signaling pathway, focal adhesion	Myopathy, glaucoma, stroke (Kuo, Labelle-Dumais et al. 2012)	3.838731
PAK7	13:111152478	rs6056921	Cell survival and neuronal growth	Learning deficits (Nekrasova, Jobes et al. 2008)	3.83302
GMDS	20:9816860	rs1013303	CNS development		−4.6509652
HK2	6:1749928	rs2229626	Glycolytic metabolism	Glioblastoma (Wolf, Agnihotri et al. 2011)	−4.2077072
COL9A1	2:75113657	rs476863	Protein digestion		3.8077398
COLEC12	6:70927967	rs2076867	Vascular endothelium function		−3.7897615
GABRG3	20:9815528	rs9672753	GABA signaling	Alcohol/psychostimulant dependence (Dick, Edenberg et al. 2004)	−3.6973569
DHRS3	18:335732	rs4618985	Cardiac tissue development	Myocardium malformation (Billings, Pierzchalski et al. 2013)	−3.6398269
DERL1	15:27631886	rs11781842	Protein folding	Alzheimer’s Disease (Honjo, Ito et al. 2012)	3.6321892
ITGA6	1:12671324	rs10930556	Cell adhesion molecule	Depression (Orsetti, Di Brisco et al. 2008)	−3.6159337
SLC8A1	7:28422523	rs4952410	Various cardiovascular	Heart failure, arrhythmia (Khananshvili 2013)	−3.6005603

Psychopharmacology (Berl). Author manuscript; available in PMC 2016 August 01.

Gene	Chromosome/Position	SNP	Pathway/Function	Disease association	|Z| score
PRKCH	7:28468319	rs2147539	Vascular smooth muscle contraction	Stroke (Kitazono, Kubo et al. 2008)	3.5958762
ENPP7	8:124028479	rs9900295			−3.5718529
BAIAP2	2:173297206	rs8067235	Neuronal cell migration and development	Neurodegenerative disease (Thomas, Foye et al. 2001), Autism (Toma, Hervas et al. 2011), ADHD (Ribases, Bosch et al. 2009)	3.8077398
CACNA2D3	20:9816249	rs3773574	Cardiovascular muscle contraction		−3.7897615
G3					
ACTB	7:5567112	rs7612	Neuronal cell migration and development	Baraitser-Winter syndrome (Eker, Derinkuyu et al. 2013), amphetamine dependence (Shibasaki, Mizuno et al. 2011)	4.5488757
CDKL1	14:50799126	rs7161563	Cell growth		−3.8761389
MAPT	17:44005821	rs1001945	Neuronal protein folding	Neurodegenerative disease (Di Battista, Pascale et al. 2013)	−3.7863989
NCOR2	12:125033338	rs10773092			−3.6777492
C7	5:40980807	rs971077			3.6414519
ORC3	6:88343318	rs9444530	Neuronal development		−3.5746469
WASF3	13:27186242	rs9507747	Neuronal development		−3.5607125
B3GALTL	13:31838688	rs1912795	Neuronal development	Peter's syndrome (Schoner, Kohlhase et al. 2013)	3.5330929
PAH	12:103298517	rs1522307		Mental retardation, seizure (Alibakhshi, Moradi et al. 2013)	−3.5131514
ATP6V0A4	7:138419932	rs2353841	Vibrio cholerae infection	Renal tubular acidosis (Elhayek, Perez de Nanclares et al. 2013)	3.4916802
KCNK10	14:88727363	rs724612	Potassium channel function	Learning deficits (Deng, Xiao et al. 2009)	−3.4080874
OXTR	3:8796547	rs11706648	Calcium signaling		3.3958792
RORB	9:77281536	rs7033059	DA/GLU signaling, circadian rhythms	Bipolar disorder (McGrath, Glatt et al. 2009)	3.3911085
PRKCQ	7:138424046	rs4620621	Vascular smooth muscle contraction		3.3772692
GXYLT1	10:6616336	rs7971943			3.357371

Gene	Chromosome/Position	SNP	Pathway/Function	Disease association	|Z| score
DAPK1	12:42529472	rs11141918	Cell death protein	Alzheimer's disease (Hainsworth, Allsopp et al. 2010)	3.3554198
KCNMA1	9:90246382	rs10437394	Vascular smooth muscle contraction		3.354954
PIK3C2B	10:78651927	rs6594014	Vascular smooth muscle contraction	Infarcted myocardium (Eun, Song et al. 2010)	−3.2890792
PRKCE	1:204449434	rs4953247	Vascular smooth muscle contraction	Substance use and cardiovascular stress (Nikpay, Seda et al. 2012)	−3.280417
ST6GALNA C32:45985386		rs407271	Cardiovascular function (Hodgkinson, Enoch et al. 2010)		3.2732021

G1, G2, G3=genetic components; SNP=single nucleotide polymorphism;

* pathways from gene card; other source of disease association and gene function is PubMed