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Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach

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Niculescu, Alexander B., III, David S. Segal, Ronald Kuczynski, Thomas Barrett, Richard L. Hauger, and John R. Kelsoe. Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. *Physiol Genomics* 4: 83–91, 2000.—We have used methamphetamine treatment of rats as an animal model for psychotic mania. Specific brain regions were analyzed comprehensively for changes in gene expression using oligonucleotide GeneChip microarrays. The data was cross-matched against human genomic loci associated with either bipolar disorder or schizophrenia. Using this convergent approach, we have identified several novel candidate genes (e.g., signal transduction molecules, transcription factors, metabolic enzymes) that may be involved in the pathogenesis of mood disorders and psychosis. Furthermore, for one of these genes, G protein-coupled receptor kinase 3 (GRK3), we found by Western blot analysis evidence for decreased protein levels in a subset of patient lymphoblastoid cell lines that correlated with disease severity. Finally, the classification of these candidate genes into two prototypical categories, psychogenes and psychosis-suppressor genes, is described.

brain; amphetamine; microarrays; GRK3

STIMULANT ADMINISTRATION in humans mimics many of the signs and symptoms of mania and psychosis. Specifically, single-dose methamphetamine administration in humans reproduces some of the core symptoms of mania: increased energy, euphoria, irritability, racing thoughts, rapid speech, hyperactivity, hypersexuality, decreased need for sleep, and psychomotor agitation (18). More prolonged administration frequently results in psychotic symptoms that resemble psychotic mania or the positive symptoms of schizophrenia (2). As amphetamines increase synaptic dopamine levels, these clinical phenomena are consistent with a large body of data arguing for the role of dopamine in mania and psychosis (56).

Attempts to map genes for these disorders by positional cloning have yielded some recent success. About 20 genomic regions have been implicated by linkage

studies, many of which are found in studies of both bipolar disorder and schizophrenia (5, 31). This is consistent with the clinical overlap observed between these two broad categories of psychiatric disorders. One of the major difficulties in fine mapping and identification of susceptibility genes for these and other complex genetic disorders is the length of the linkage peaks, which are typically 20 cM or greater and may contain hundreds of genes.

The advent of microarray technologies, on the other hand, has recently offered an approach capable of simultaneously examining the expression of thousands of genes. Again, however, there is a degree of uncertainty regarding which of the numerous genes that show changes in an animal model experimental paradigm are directly germane to the human disease process. We attempted to solve the above two problems by intersecting the two approaches. We reasoned that this would cross-validate the findings of each method, reduce the uncertainty inherent in the two approaches, and identify a limited number of high-probability candidates. We have termed this approach “convergent functional genomics.”

We report here that the gene expression and linkage results converge on a series of novel high-probability candidate genes for mania and psychosis. These candidate genes may also play a role in the pathophysiology of stimulant abuse and dependence, a major public health problem in the United States and throughout the world. Finally, we describe their possible grouping into two prototypical classes, psychogenes and psychosis-suppressor genes.

MATERIALS AND METHODS

Methamphetamine treatments in rats. The single-dose methamphetamine treatment experiments described below were done twice, independently, with different sets of animals, at different times, to assess reproducibility.

Three Sprague-Dawley rats were treated with 4.0 mg/kg methamphetamine, and three rats were treated with normal saline injection. Twenty-four hours later the brains were harvested and specific brain regions [prefrontal cortex (PFC), amygdala (AMY)] were dissected out on a refrigerated dissection block. The dissections were accomplished as described previously by first slicing the brain into defined coronal slices (51). PFC was then dissected as whole fore-

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brain anterior to the corpus callosum excluding the olfactory bulb (45). AMY was included in a block dissected freehand from a slice extending from 2 mm posterior to 5 mm posterior to bregma according to a brain atlas (45). The dissections were conducted so as to be inclusive of the entirety of these dopamine terminal regions. The tissue samples were flash frozen in liquid nitrogen and stored at -70°C until the time of gene expression analysis. The PFC and AMY were chosen as key components of the limbic system putatively involved in the cognitive and emotional aspects of amphetamine action, respectively (52).

GeneChip experiments. We employed the Affymetrix U34A chip, which measures 7,000 cDNAs and 1,000 expressed sequence tags (ESTs). As mentioned, two independent amphetamine treatment experiments were assayed. The analysis was carried out at the UCSD/VA Medical Center GeneChip Core Facility.

Samples were handled according to the recommendations of Affymetrix (Santa Clara, CA), the manufacturer of GeneChip microarrays. Total RNA was isolated from tissue using standard protocols, comprised of STAT-60 extraction buffer and phenol/chloroform extractions. An aliquot of RNA was examined by gel electrophoresis for quality and lack of degradation. cDNA was synthesized, followed by the generation of biotin-labeled antisense cRNA by an *in vitro* transcription reaction using the cDNA as a template. After fragmentation, the cRNA hybridization cocktail was prepared, cleaned, and applied to the Affymetrix GeneChip oligonucleotide array overnight in a GeneChip hybridization oven. Immediately following hybridization, the probe array was washed and then stained with a streptavidin-phycoerythrin (SAPE) fluorescence tag. The GeneChip Fluidics Station automates the washing steps, which remove nonspecifically bound cRNA and stain. Once the probe array had been hybridized, stained, and washed, it was scanned using a Hewlett-Packard GeneArray scanner. A GeneChip Operating System, running on a PC workstation, controlled the functions of the scanner and collected fluorescent intensity data. Data was processed using GeneChip expression analysis software from Affymetrix.

Data analysis. Standard default settings of the Affymetrix GeneChip Expression Algorithm were used for our analysis. A gene had to be called "present" and "changed" in at least one of two experiments and had to have an "average difference change" greater than 50 and a fold change greater than 2.0 in two of two experiments (D. Lockhart, personal communication). An increase or decrease of twofold was chosen as a conventional empirical cutoff for changes in gene expression. The genes that were induced more than twofold in both experiments were identified by their GenBank accession numbers. Their human homologs and human chromosomal map locations were then determined using the National Center for Biotechnology Information database. GeneCard (Weizmann Institute), a comprehensive database containing all the various information available regarding known genes and their functions, was also used for each gene identified by our screen. Induced genes were then matched to the published genomic loci for bipolar disorder and schizophrenia (5, 31) as a way of cross-validating our results and identifying high-probability candidate genes. To be considered close to a genomic hotspot, the gene had to map to within 10 cM of a marker for which at least suggestive evidence for linkage had been reported. The Marshfield integrated linkage map was used as a reference for genetic location.

Western blot analysis. Six patients with bipolar disorder and six normal controls were selected from the UCSD Bipolar Genetics Study cohort. All patients and controls were inter-

viewed using the Structured Clinical Interview for DSM-IV, and diagnoses were made by a best estimate procedure as described previously (33). All subjects were white, of North American origin. Each bipolar subject came from a family with an LOD score >0.3 at D22S419 on chromosome 22. Subject lymphocytes had been previously immortalized using Epstein-Barr virus and cultured under identical conditions and to a similar degree of expansion prior to being frozen in liquid nitrogen. Cells were thawed simultaneously and grown in RPMI medium with 10% FBS in a 37°C incubator with 5% CO_2 , to a density of 1×10^6 cells/ml. Equal amounts of total protein (100 μg), as determined by the Bradford method, were loaded into each lane. The cells were lysed in lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 10 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 10 $\mu\text{g}/\text{ml}$ benzamide, 10 $\mu\text{g}/\text{ml}$ leupeptin, 10 U/ml soybean trypsin inhibitor, 5 $\mu\text{g}/\text{ml}$ aprotinin, 1 $\mu\text{g}/\text{ml}$ pepstatin A, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 1 mM NaF). Total cell lysates of 100 μg were resolved by SDS-PAGE on a 7% precast gel (NuPAGE, Invitrogen-Novex) and transferred to polyvinylidene difluoride membranes (PVDF, Invitrogen-Novex). The blot was incubated in the primary antibody at 4°C overnight (anti-GRK3 goat polyclonal IgG, E-15, sc-9306, Santa Cruz, 1/200 dilution) and then with a horseradish peroxidase-conjugated secondary antibody (anti-goat HRP, sc-2033, Santa Cruz, 1/5,000 dilution) for 1 h. The bound antibodies were visualized by enhanced chemiluminescence (Amersham). The specificity of the antibody was verified by a separate Western analysis using purified GRK2 and GRK3 protein.

RESULTS

We used the criterion of at least a twofold change in each of two independent animal experiments to select those genes with the most robust and reproducible change in expression. Genes meeting these criteria are summarized in Table 1 for the PFC and in Table 2 for the AMY. The chromosomal location of the human homologs of these genes was then compared with published reports of linkage for bipolar disorder and schizophrenia, as well as our own unpublished data. Genes were considered positional candidates if they mapped to within 10 cM of a marker for which there was at least one report of suggestive evidence of linkage (39). As shown in Tables 1 and 2, eight of these genes met these criteria. For six of these a plausible biological hypothesis can be constructed regarding their role in pathophysiology. These six genes which are implicated by a convergence of data from both amphetamine response and clinical linkage studies are compelling and novel candidates for disease susceptibility loci. Their roles and map locations are described below.

G protein-coupled receptor kinase 3. G protein-coupled receptor kinase 3 (GRK3) mediates homologous desensitization for a variety of neurotransmitters by phosphorylation of G protein-coupled receptors (GPCRs). GRK3 maps to human chromosome 22q11. This region had been previously implicated in bipolar disorder by our group (32, 38) and others (11, 15). In fact, 22q yielded the highest LOD scores of any chromosomal region in our genome survey (results to be reported

Table 1. *Candidate genes reproducibly induced in the prefrontal cortex*

Accession No. (rat/human)	Gene Symbol	Description	Fold Induction	Human Chromosomal Location	Linkage Region
M87855/NM005160	GRK3	G protein-coupled receptor kinase 3	14.2*	22q11	B
J03179/U48213	DBP	D-box binding protein	7.0	19q13.3	B
M95591/X69141	FDFT1	Farnesyl-diphosphate farnesyltransferase 1	2.9	8p23.1-p22	S
AF090134/AF173081	MALS-1	Vertebrate LIN7 homolog 1	2.9	12q21.3	B

"Fold Induction" is average of two independent biological experiments; each experiment pooled tissue from 3 methamphetamine-treated and 3 control rats. *An indication of the specificity of the result is that GRK2, its close homolog, demonstrated no change in expression in either experiment (fold changes of 1.1 and 1.0 in the two experiments). A gene was scored as mapping to a linkage region for either schizophrenia (S) or bipolar disorder (B) if its human homolog mapped to within 10 cM of a marker for which at least suggestive evidence of linkage had been reported.

separately). Consistent with many findings in this field, this linkage peak was broad and spanned nearly 20 cM. One of the highest LOD scores in this region was 2.2 at D22S419, which maps to within 40 kb of GRK3. This marker is also quite close to the markers identified in the two other independent positive linkage reports for 22q in bipolar disorder. A marker within the GRK3 gene, D22S315, has also been implicated in a study of eye tracking and evoked potential abnormalities in schizophrenia (44).

The known physiological role of GRK3, described in more detail below, suggests the hypothesis that a defect in its function could lead to supersensitivity to dopamine or a defect in the homeostatic adaptation to dopamine, which in turn could predispose to illness. To test this hypothesis, we examined levels of GRK3 protein in lymphoblastoid cell lines of individuals with bipolar disorder from the families in our genome scan with the strongest evidence of linkage to 22q11. Consistent with this model, as shown in Fig. 1, four of six such subjects demonstrated reduced expression of GRK3. Furthermore, the four subjects that showed a decrease had a more severe form of bipolar disorder (bipolar I), whereas the subjects that did not show a decrease in GRK3 had a milder form of bipolar disorder (bipolar II). These data suggest that a defect in transcriptional regulation in GRK3 may contribute to the susceptibility to bipolar disorder in a subset of individuals. As only a subset of families in our collection

showed evidence of linkage to 22q11, only a limited number of subject cell lines were available for examination. Furthermore, although every effort was made to control for cell culture conditions, such measures in a clinical sample are vulnerable to some variability. Therefore, these results must be qualified and considered suggestive. However, they do raise an intriguing hypothesis that warrants testing in a larger sample, including efforts to identify a genomic basis for such a possible difference in gene expression and to measure its functional impact.

D-box binding protein. D-box binding protein (DBP) is a clock gene that maps to chromosome 19q13.3. Chromosome 19 has not been a strong linkage region for psychiatric disorders; however, one study has implicated this region in a large Canadian kindred with bipolar disorder (43). In this sample, D19S867, which is ~2 cM from DBP, yielded a LOD score of 2.6.

Farnesyl-diphosphate farnesyltransferase 1. Farnesyl-diphosphate farnesyltransferase 1 (FDFT1) is an enzyme that mediates one of the first steps of cholesterol biosynthesis. It is located on 8p23.1-p22 near the telomere. Numerous studies have implicated 8p in both schizophrenia and bipolar disorder; however, most of these results are about 40–50 cM centromeric to FDFT1. Two studies have reported evidence for linkage to schizophrenia within 10 cM of FDFT1. Wetterberg et al. (55) have reported a LOD score of 3.8 at D8S264 in a large Swedish isolate. The National Insti-

Table 2. *Candidate genes reproducibly induced in the amygdala*

Accession No. (rat/human)	Gene Symbol	Description	Fold Induction	Human Chromosomal Location	Linkage Region
AA799479/AF038406	NDUFS8	NADH-coenzyme Q reductase	20.8	11q13	
L19998/L19999	SULT1A1	Sulfotransferase 1A1	4.3	16p12.1-p11.2	B
AB017711/Z27113	POLR2F	RNA polymerase II polypeptide F	3.9	22q13.1	B,S
X14323/U12255	FCGRT	IgG Fc receptor transporter- α	3.2	19q13.3	B
M81183/X57025	IGF-I	Insulin-like growth factor I	3.0	12q22-q24.1	B
AA998683/(AJ224874) EST*	HSPB1	Heat shock 27-kDa protein 1	2.8	7q22.1	
S62933/U05012	NTRK3	Neurotrophin receptor 3	2.7	15q25	
X59249/L77730	ADORA3	Adenosine receptor A3	2.7	1p21-p13	
U64689/U69140	FEZ2	Fasciculation and elongation protein- ζ 2 (zygin II)	2.3	2p22	

"Fold induction" is average of two independent biological experiments; each experiment pooled tissue from 3 methamphetamine-treated and 3 control rats. A gene was scored as mapping to a linkage region for either schizophrenia (S) or bipolar disorder (B) if its human homolog mapped to within 10 cM of a marker for which at least suggestive evidence of linkage had been reported. *The putative human homolog for this expressed sequence tag (EST).

Table 3. *Candidate psychogenes and psychosis-suppressor genes*

Psychogenes	Psychosis-Suppressor Genes
DBP	GRK3
FDFT1	SULT1A1
MALS-1	
IGF-I	

tute of Mental Health schizophrenia genetics consortium also reported evidence implicating a broad area of 8p in African-American pedigrees. This included two putative peaks, with one at D8S264 (NPL Z score 2.3) (30).

Vertebrate LIN7 homolog 1. Vertebrate LIN7 homolog 1 (MALS-1 or VELI1) is a cytoplasmic protein associated with glutamergic neurotransmission. It maps to 12q21.3 in a region implicated in several studies of bipolar disorder. This region was first reported in bipolar disorder through observation of a Welsh family in which bipolar disorder and Darier's disease cosegregated (10). Although the Darier's region is somewhat distal to MALS-1, Morissette et al. (43) have reported evidence of linkage of bipolar disorder to markers on 12q, with a maximum at D12S82 (Z_{all} 4.0, LOD score 2.2), which is ~2 cM from MALS-1.

Sulfotransferase 1A1. Sulfotransferase 1A1 (SULT1A1) inactivates dopamine and other phenol-containing compounds by sulfation. It has not yet been precisely mapped, but cytogenetic data locate it to chromosome 16p12.1-p11.2, near a genomic locus implicated in bipolar disorder (D16S510, LOD score 2.5) (17) and alcohol dependence (D16S675, LOD score 4.0) (20).

Insulin-like growth factor I. Insulin-like growth factor I (IGF-I) has been shown to regulate tyrosine hydroxylase expression and is located on chromosome 12q22-q24.1. It is at a map position of 109 cM, 13 cM telomeric to MALS-1, and in the same 40 cM region described above. This region is implicated in bipolar disorder and extends from D12S82 at 96 cM (NPL Z_{all} 4.0) (43) to PLA2 at 136 cM (LOD score 2.49) (10).

DISCUSSION

The present study of 8,000 genes found a number of genes upregulated in the PFC (GRK3, DBP, FDFT1, and MALS-1) and AMY (SULT1A1 and IGF-I) of rats 24 h after a single methamphetamine injection. These genes were identified as particularly interesting because they were both upregulated in this animal model of mania and they mapped to regions implicated in bipolar disorder in humans. These data indicate the utility of microarray gene profiling studies in animal models of psychiatric disorders and the power of this approach for identifying positional candidate genes for complex genetic disorders.

Physiological roles of the genes. The six genes identified through this approach (Table 3) each have biological functions that make them intriguing novel candidate genes for bipolar disorder and schizophrenia.

Several of these genes have roles in modulating, among other things, dopamine neurotransmission. The known physiological role of GRK3 in desensitization of receptors and its map location make it one of the more interesting candidates identified in this study. In the continuing presence of high agonist concentrations, GPCR signaling is rapidly terminated by a process termed "homologous desensitization." Homologous desensitization of many agonist-activated GPCRs begins when GRKs phosphorylate serine and threonine residues on the receptor's cytoplasmic tail and/or third intracellular loop (46). The consequent binding of β -arrestin to phosphorylated GPCRs decreases their affinity for cognate heterotrimeric G proteins, thereby uncoupling the receptor from the $G\alpha$ subunit by steric hindrance. Dopamine D_1 receptors can be phosphorylated and desensitized via a GRK3 mechanism (53), and GRK3 expression is particularly high in dopaminergic pathways in the central nervous system (3). These data are consistent with our results in that GRK3 may exert an important regulatory effect on brain dopamine receptors. Because dopamine receptors play an important role in amphetamine's action in the brain, we hypothesize that amphetamine-induced upregulation of GRK3 counterregulates dopamine receptor signaling initiated by mesocorticolimbic dopamine release as a way to maintain homeostasis. GRK3 has also been shown to play a role in the desensitization of β -adrenergic receptors and corticotropin-releasing factor (CRF) receptors and may mediate desensitization for a variety of different neurotransmitter receptors (23).

These data suggest that a major physiological role for GRK3 in neurons may be to act as a brake for signal transduction for some GPCRs. A defect in GRK3 function could lead to an inability to desensitize receptors, and hence, a heightened responsiveness to dopamine and other neurotransmitter signals in the brain. Such genetic variation might influence individual variation in behavioral sensitization to stimulants in animals or humans. One could predict that individuals predisposed to mania might have either low levels of the normal protein or high levels of mutated hypoactive

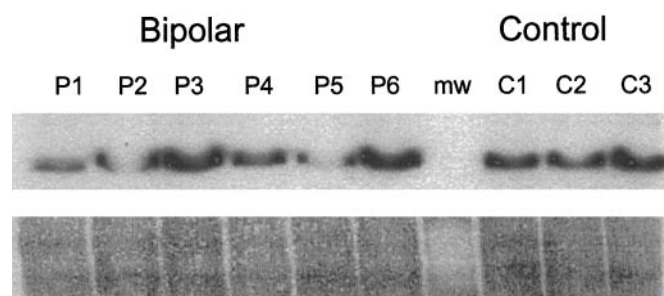


Fig. 1. GRK 3 protein expression levels in human lymphocytes from bipolar members of families with evidence of linkage to chromosome 22q11 (bipolar) and normal controls (control). *Top:* Western blot with an antibody specific for GRK3 (sc-9306). *Bottom:* gel total protein, Coomassie stain. Four of six subjects demonstrate a reduction in GRK3 expression. The subjects that showed a decrease had a clinically more severe form (bipolar I) than the subjects who did not show a decrease (bipolar II); mw, molecular weight standards lane.

protein. Conversely, individuals susceptible to depression might have either high levels of the normal protein or normal levels of mutated hyperactive protein. Our Western blot results (see above, and Fig. 1), are consistent with this model. This model also is supported by postmortem studies in people who had depression that led to suicide and who had increased levels of GRK2/3 protein in their PFC (22).

SULT1A1 is a sulfotransferase that inactivates dopamine and other phenol-containing compounds by sulfation. It potentially plays a role in limiting the neuronal stimulatory and psychosis-promoting effects of dopamine. Although it is not a primary regulator of synaptic dopamine concentration, a defect in this gene could lead to impaired clearing of dopamine from the extracellular space, with a resulting amphetamine-like effect.

IGF-I stimulates increased expression of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine (25). It has also been shown to have trophic effects on dopamine brain neurons and to protect dopamine neurons from apoptotic death (35). IGF-I also induces phosphatidylinositol 3-kinase survival pathways through activation of AKT1 and AKT2; it is inhibited by tumor necrosis factor (TNF) in its neuroprotective role. IGF-I gene disruption in mice results in reduced brain size, central nervous system hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons (4). Defects of IGF-I in humans produce growth retardation with deafness and mental retardation.

In addition to dopaminergic neurotransmission, these genes highlight several other physiological mechanisms and biochemical pathways potentially involved in bipolar disorder and schizophrenia. DBP is a CLOCK-controlled transcriptional activator (48) that shows a robust circadian rhythm. In experiments done in mice (58), its highest expression in the brain is in the suprachiasmatic nucleus (SCN), but it is also present in the cerebral cortex and caudate-putamen. In the SCN, DBP mRNA levels showed a peak at early daytime (ZT/CT4) and a trough at early nighttime (ZT/CT16) in both light-dark and constant dark conditions. In the cerebral cortex and the caudate-putamen, DBP mRNA was also expressed in a circadian manner, but the phase shift of DBP mRNA expression in these structures showed a 4- to 8-h delay compared with the SCN. These data implicate DBP as an arm of the circadian clock. DBP knockout mice show reduced amplitude of the circadian modulation of sleep time, as well as a reduction in the consolidation of sleep episodes (21). Clock genes have been shown to be essential for the development of behavioral sensitization to repeated stimulant exposure (1). Circadian rhythm abnormalities have also been implicated in mood disorders (37), and stimulant exposure disrupts circadian rhythms and abrogates sleep (8). Taken together, the connections between clock genes, stimulant sensitization, circadian rhythmicity, sleep, and mood disorders make DBP a compelling candidate gene for bipolar disorder.

FDFT1 is the first step of sterol biosynthesis uniquely committed to the synthesis of cholesterol (49). As such, it has received attention as a target for the development of cholesterol-lowering drugs. Interestingly, primary prevention human trials have shown a correlation between lowering cholesterol and suicide, postulated to occur because of a reduction in the number of serotonin receptors in synapses (16). Studies in monkeys have also shown an association between cholesterol and central serotonergic activity (28). Mice homozygously disrupted for the squalene synthase gene exhibited embryonic lethality and defective neural tube closure, implicating de novo cholesterol synthesis in nervous system development (54). Moreover, de novo cholesterol synthesis was shown to be important for neuronal survival, and apoE4, which is a major risk factor for Alzheimer's disease, has been implicated in inducing neuronal cell death through the suppression of de novo cholesterol synthesis (42). As such, neuronal cholesterol synthesis, of which squalene synthase is a key regulator, may correlate positively with both elevated mood and neuronal survival.

MALS-1 is a PDZ domain containing cytoplasmic protein that is enriched in brain synapses, where it associates in complexes with PSD-95 and *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (26). It has been implicated in regulating the recruitment of neurotransmitter receptors to the postsynaptic density (26), as well as being part of a complex with CASK and Mint 1 that couples synaptic vesicle exocytosis to cell adhesion (9).

Two additional genes met our criteria of reproducibility and mapping to a linkage region, but their known functions so far make them less obvious disease gene candidates. RNA polymerase II polypeptide F (POLR2F) maps to 22q13.1 at ~10 cM distal to D22S278, which has been implicated in several studies of both bipolar disorder and schizophrenia as described above. POLR2F is responsible for mRNA production and may control cell size (50) and overall body morphological features (6). It is more active in metabolically active cells (50). FCGRT is a receptor for the Fc component of IgG that structurally resembles the major histocompatibility complex class I molecule (27). FCGRT maps to 19q13.3 near DBP and near a marker implicated in bipolar disorder as described above.

Several other genes did not meet our stringent criteria but nevertheless deserve mention. Fibroblast growth factor receptor 1 (FGFR1) had an average fold change of 4.1, although the increase was only 1.8-fold in one of the two experiments. Increased expression of astrocytic basic FGF in response to amphetamine has been previously demonstrated (19). Furthermore, FGF-2, a ligand for FGFR1, has been shown to regulate expression of tyrosine hydroxylase, a critical enzyme in dopamine biosynthesis (47). FGFR1 maps to chromosome 8p11.2-p11.1, ~10 cM centromeric to a genomic locus near D8S1771 (8p22-24), which demonstrated evidence of linkage to schizophrenia in several studies (7, 34, 40). Heat shock 27-kDa protein 1 (HSP27, HSPB1) has been implicated in stress-resistance re-

sponses in a variety of tissues. It may play a role in promoting neuronal survival (41) and may be induced in brain by kainic acid-induced seizure (29). HSPB1 maps to 7q22.1 at ~20 cM from a region implicated in bipolar disorder in two independent samples (11, 12).

Animal model-critical aspects. Amphetamine treatment protocols have been used as rat models of substance abuse and psychiatric disorders for over three decades. We view three choices in experimental design to be critical: treatment regimen, time point after drug administration, and brain regions to be studied.

First, in terms of amphetamine treatment regimen, rats were treated with a single dose of methamphetamine (4.0 mg/kg) as an animal model of mania and psychotic mania. In humans, single-dose treatment is associated with the symptoms that most closely resemble mania: increased energy, euphoria, irritability, racing thoughts, rapid speech, hyperactivity, hypersexuality, decreased need for sleep, and psychomotor agitation. Over time, patients with mania develop psychotic symptoms that resemble some of the positive symptoms of schizophrenia. Chronic treatment, therefore, more closely resembles psychotic mania. Methamphetamine, a derivative of amphetamine, was chosen for two reasons. First, it is the most commonly abused form of amphetamine in humans and the fastest growing drug of abuse in the United States. Second, it leads to more sexual activation, a key symptom of mania, than amphetamine proper (data to be reported separately).

Second, harvesting the brain 1 h after the (last) amphetamine dose, as has been a common practice in the field, may reveal a very high number of changed transcripts, many of them related to a less specific early response. Harvesting later, however, likely offers a window on gene expression where a lower, more manageable, number of transcripts in general are changed. We chose a 24-h time point as more likely to detect changes of relevance to mania and psychosis. We reasoned that at 24 h, most short-term gene induction relevant to acute intoxication and behavioral activation would have subsided. Furthermore, 24 h after a single moderate to high dose, animals already exhibit a sensitized response to a second amphetamine challenge. As mania and psychosis are typically chronic processes in humans, more persistent gene changes are more likely to be central to pathophysiological mechanisms.

Third, we chose to focus initially on the PFC and AMY. These brain regions were chosen based on the extensive literature demonstrating their central role in cognition and emotion (24, 36). The PFC in particular has been shown to be activated in brain imaging studies of mania and depression. Specifically, the subgenual region of the PFC is important for mood states (13). This region has extensive connections with other regions involved in emotional behavior, such as the AMY, the lateral hypothalamus, the nucleus accumbens, and the noradrenergic, serotonergic, and dopaminergic systems of the brain stem. It is also of interest that functional imaging studies in humans have shown

this region to be activated by sleep deprivation (14, 57). This is consistent with clinical data that show that sleep deprivation has a rapid antidepressant effect and can induce hypomania. Moreover, amphetamine is in a way a pharmacological anti-sleep agent. Together these data argue that dopamine activation in the PFC is a mechanism common to regulation of mood and sleep and the antidepressant action of sleep deprivation. Our identification of one of the circadian clock components, DBP, as a candidate gene provides a possible molecular underpinning for these physiological connections.

Evaluation of results. It is striking that we identified only a very small number of genes that showed reproducible change. There are at least two reasons for that. One reason is by design; we had hypothesized that by looking at gene expression changes 24 h after the methamphetamine dose, we would see a reduced number of more specific, late-response genes induced. The second reason is that our litmus test for reproducibility was repeating the animal experiments de novo, independently, at a different time. The number of biological and nonbiological variables in such a complex experiment is vast. A number of transcripts that mapped to genomic loci of interest showed a significant change in one experiment but not in the other. We chose a threshold of at least twofold induction to identify the genes most robustly induced. A lower threshold might still detect genes with physiologically meaningful changes and result in a greater reproducibility between experiments.

The second observation is that the transcripts that we have deemed so far to show reproducible changes by our comprehensive criteria were all induced. It may be that the dynamic range for detecting decreases is compressed, especially since many of the brain-expressed genes of interest are low-abundance transcripts. A decrease in the experimental sample as opposed to the baseline control may make a transcript go below the threshold of detection and be called absent by the GeneChip Expression Analysis Algorithm.

The third observation is that a major fraction of the genes that show reproducible changes in gene expression map in the proximity of genomic loci thought to be involved in bipolar disorders or schizophrenia. This is an unexpectedly strong validation of our experimental design. Although by no means direct proof, the convergence of the animal model results and the human genetic data positions the genes identified by us as strong candidates for direct involvement in the pathophysiology of both amphetamine abuse and psychiatric disorders. Given the number of genomic regions that have been implicated in bipolar disorder and schizophrenia, it is important to evaluate the probability that some of the genes we identified mapped to a disease locus by chance. We required that a gene map to within 10 cM of a marker identified in at least one study as having suggestive evidence of linkage. As the average genomic region meeting these criteria is 30 cM long, and ~20 such regions have been reported, then about 20% of the genome is implicated in bipolar disorder or

schizophrenia (5, 31). Therefore, there is about a 1-in-5 probability that a gene falls within a putative linkage region by chance alone. However, our animal model gene expression studies identified about 1 in 1,000 genes as being changed. This leads to an estimated probability of 1 in 5,000 that a gene would meet both criteria. For an estimated genome size of about 100,000 genes, that leads to about 20 genes per genome meeting both criteria, which is roughly the number of linkage hotspots. Our approach thus identifies about one gene per hotspot. Clearly, the above estimation involves a series of assumptions and approximations. Not all the genes induced by methamphetamine are necessarily involved in the susceptibility to illness, and some susceptibility genes may not be induced by methamphetamine. Definitive identification of disease genes will require the discovery of a polymorphism of functional significance and its association with illness. This will likely require large-scale sequencing of both coding and noncoding regions in numerous affected individuals. Nevertheless, our approach arguably dramatically narrows the field and identifies a series of high-probability candidate genes for future in-depth study.

Grouping candidate genes in classes: psychogenes and psychosis-suppressor genes. Based on the biological roles of the candidate genes we have identified so far, and by analogy to cancer biology, we propose that genes involved in psychiatric disorders can be viewed as falling into two prototypical categories. Genes whose activity promotes processes that lead to mania or psychosis could be called "psychogenes," by analogy to oncogenes. Conversely, genes whose activity suppresses processes that lead to these psychiatric disorders could be called "psychosis-suppressor" genes, by analogy to tumor suppressor genes (Table 3). Although such a classification is inevitably simplistic, we believe that it has heuristic value for psychiatric illness, as it has for cancer biology. This classification may be useful in considering the role of these putative disease genes in pathophysiology and as targets for therapeutic intervention.

Insights into polygenic disorders. It is possible that genes that show concomitant changes in expression levels (Tables 2 and 3) may be interacting pathophysiologically and merit further analysis as coacting gene groups. The concept that "genes that change together act together" provides a simple testable model for unraveling complex polygenic diseases like bipolar disorder, schizophrenia, and others.

Conclusion. In conclusion, we have identified a series of high-probability candidate genes for mania and psychosis using the convergence of animal model data and human genetic linkage data. These targets warrant detailed study for genomic variation in clinical populations and behavioral variation in knockout animal models. Lastly, although complex genetic disorders have been challenging problems to address through positional cloning alone, the convergent approach described in this report may be more generally applicable to a variety of other polygenic diseases.

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