Convergent functional genomics: application to bipolar disorder

Alexander B Niculescu III and John R Kelsoe

The recent development of microarray technologies has made possible the simultaneous measurement of mRNA levels for thousands of genes and a new genomic method termed gene expression profiling. The application of this approach to animal models or post-mortem tissue provides a powerful tool for the discovery of novel genes involved in psychiatric disorders. This approach has strengths that are complementary to those of another genomic method for gene discovery, positional cloning. Microarray technologies and their application to post-mortem tissue and animal models of bipolar disorder are reviewed. A novel approach termed convergent functional genomics, which integrates gene profiling and positional cloning in order to rapidly identify candidate disease genes, is also described.

Keywords: bipolar disorder; functional genomics; gene expression profiling; mania; manic-depressive illness; psychosis; microarrays.

Ann Med 2001; 33: 263–271.

Introduction

Bipolar disorder or manic-depressive illness is a common psychiatric disorder that affects approximately 1% of the population worldwide (1). It is characterized by alternation between two extremes of mood state: mania and depression. In mania, patients experience euphoria or irritability, increased energy and activity, grandiosity, decreased need for sleep, racing thoughts, rapid speech and risk taking. Depression is the opposite of mania with symptoms of sadness, lack of motivation, loss of pleasure, insomnia or hypersomnia, appetite change, feelings of worthlessness and suicidal ideation. Psychosis can occur in either of these states, and the lifetime suicide rate is approximately 17%. There is an enormous variety in the way in which these two states alternate over the course of a person's life. Although there are many effective treatments, the lack of a fundamental understanding of pathophysiology has significantly hindered the discovery of novel therapeutic mechanisms and targets for drug action.

Numerous family, twin and adoption studies have argued for a strong genetic contribution to the aetiology of bipolar disorder (2). Based on these data, much effort has recently focused on the use of positional cloning and linkage strategies to identify susceptibility genes. These studies are reviewed elsewhere in this issue. Although this approach has great promise to elucidate the fundamental mechanisms of this disorder, as with other complex genetic traits, linkage studies in psychiatry have turned out to be very arduous. This is likely due to the polygenic nature of most psychiatric disorders. Furthermore, psychiatric phenotypes are complex and lack the clear quantitative measures and endophenotypes available for other comparably complex polygenic disorders, such as diabetes or hypertension. Nevertheless, the accumulation of larger family sets and independent genome scans has led to the identification of several arguably reproducible linkage peaks. However, a major problem that is consistent with other complex disorders is that these linkage peaks are broad and may contain hundreds, or even thousands, of genes (3). This approach by itself does not permit easy identification of specific disease genes.

The recent development of DNA microarray technology in combination with the identification of most genes in both human and rodent genomes provides an alternative, comprehensive genomic approach to the identification of disease genes and genetic mechanisms of disease. In this review, we will describe microarray technologies and their potential application to the genetics of bipolar disorder. Animal models proposed as relevant to bipolar disorder will be discussed. We will also describe one approach to the integration of this technology with positional cloning which we have termed *convergent functional genomics*.

From the Department of Psychiatry, University of California, San Diego and San Diego VA Healthcare System, La Jolla, CA, USA.

Correspondence: Alexander B Niculescu, III, MD, PhD, John R Kelsoe, MD, Department of Psychiatry, 0603, UCSD, La Jolla, CA 92093, USA. E-mail: aniculescu@ucsd.edu, jkelsoe@ucsd.edu, Fax: +1 858 5345524.

DNA microarrays

Gene expression studies in different brain regions in animal models of psychiatric disorders or in post-mortem brain have provided a powerful tool to study potential candidate genes involved in bipolar disorder. Until recently, the scope of those studies was limited to looking at specific known individual genes by *in situ* hybridization, or other modalities of quantifying mRNA. The advent of subtractive hybridization or differential display techniques permitted the transition to a more discovery-based approach, not limited in scope to known genes. This has come to full bloom with the development and increasing use in the last two years of microarray technology, which permits the simultaneous assessment of gene expression changes in thousands of genes. This approach has been termed gene expression profiling.

DNA microarray technology involves the placement of thousands of DNAs on a slide or chip at a microscopic scale such that thousands of DNAs may be arrayed within several square centimetres. This technology is currently rapidly evolving, but two basic approaches predominate. In one of these, full-length cDNAs are spotted onto glass slides by using robots. In the other, oligonucleotides are synthesized on glass slides in a massively parallel fashion. For both approaches mRNA is isolated from the tissue to be studied, fluorescently labelled and hybridized to the slides. After washing away the nonspecifically bound probe, the levels of mRNA for each gene to be interrogated is determined by microscopic quantification of the fluorescent signal. In the robotic spotting approach, mRNA from two tissues are typically labelled with different fluorescent dyes and hybridized together. The ratio of the two fluors then provides a controlled comparison of expression levels in the two tissues. The oligonucleotide-based method has been commercially implemented by Affymetrix (Santa Clara, CA). In this approach, RNA from one tissue is typically hybridized to a chip. Numerous oligos on the chip provide controls for standardization between chips and for absolute quantification of the mRNA levels.

Each of these approaches has its relative merits. Robotic spotting of full-length cDNAs is typically more economical after an initial outlay for the cDNA library and for equipment. It is also more flexible as the slides can be made locally. However, it is less able to distinguish members of gene families with substantial sequence homology. The oligo-based method is easier to implement, but more costly per chip. However, it has a high degree of standardization, and as multiple oligos are employed for each gene, it is better able to distinguish among gene family members. The completion of the human and mouse genome, and continuous miniaturization and standardization, will undoubtedly lead to further changes in this technology and will essentially permit the expression profiling of the full complement of genes in a single experiment. Powerful as this may be, the caveat for these methods is that hundreds or even thousands of genes may show changes in expression, and these data display a high degree of variability. Statistical methods are only now being developed to aid in interpretation of these results. Furthermore, this approach by itself again does not permit one to unequivocally identify which specific genes play a primary role in the disorder, and which are just secondary in nature.

This is a rapidly evolving technology that will require time to mature. However, DNA microarrays have already generated a lot of interest, and have started to be used successfully in developmental biology, cancer biology, and, more recently, in neuropsychiatric disorders (4–7). Two exciting possible applications of this technology to psychiatric illness include the study of patterns of gene expression in animal models and in post-mortem brain. A review of some proposed animal models of bipolar disorder indicates both the promise and limitations of such an approach.

Key messages

- Although highly heritable, like many other complex genetic disorders, bipolar disorder has proven a challenging problem for positional cloning because of heterogeneity, small gene effects and broad linkage peaks.
- Gene expression profiling uses microarray technology to simultaneously measure levels of expression of thousands of genes and thereby provides a genomic approach that is complementary to positional cloning.
- Results from expression profiling of animal models and post-mortem tissue can be combined with those from linkage studies in a approach termed convergent functional genomics to greatly accelerate the process of identifying candidate genes.

Animal models of bipolar disorder

As many molecular genetics and histochemical experiments are not feasible in humans, the use of good animal models is critical. We will discuss some of the models that have been used to date and also the choice of animal species to which to apply these models.

Ouabain

Mania and bipolar depression have been associated with a decrease in the activity of the sodium and potassium-activated adenosine triphosphatase (Na,K-ATPase) membrane pump. Based on this, treatment with ouabain, a Na,K-ATPase inhibiting compound, has been proposed as a pharmacological model of bipolar disorder (8–10). This model may be of some interest, as ouabain induces a hyperlocomotor phenotype and its actions are prevented by lithium pretreatment. Few studies have explored the possible role of these genes in bipolar disorder. However, some support comes from one recent report of reduction of the α2 isoform of the enzyme in post-mortem brain of subjects with bipolar disorder (11).

Sleep deprivation

The clinical observation of an antidepressant effect of sleep deprivation has prompted the examination of this phenomenon in rats as a potential animal model for mania. Sleep deprivation in man is notable for having an almost immediate effect, in contrast to other pharmacological treatments that typically require 2–3 weeks. In bipolar disorder, sleep deprivation can trigger an overnight dramatic 'switch' from depression into mania. Sleep deprivation in rats led to hyperactivity, irritability, aggressiveness, hypersexuality and stereotypy, and was reversed by haloperidol or lithium pretreatment (12). The cohort of symptoms induced by sleep deprivation does mimic those present in idiopathic mania, which make this an interesting model. However, the somewhat transient nature of the effect and the methodological complexity may have led to its limited use thus far in experimental animal studies.

6-Hydroxydopamine

Intraventricular injection of 6-hydroxydopamine (6-OHDA) leads to hyperreactivity and irritability in rats, changes that were prevented by lithium pretreatment or electroconvulsive therapy (13). The resulting hyperreactivity was proposed by the authors as a possible pharmacological model of mania. 6-OHDA treatment has been shown to destroy terminals but not cell bodies of norepinephrine (noradrenaline), and sometimes dopamine neurones. This model which may produce a hypodopaminergic state, seems in conflict with others such as amphetamine, described below, which induce a hyperdopamine state. The implications of this are not clear, but may imply a complex dysregulation of dopamine and norepinephrine systems.

Amphetamine administration

Stimulant administration in man mimics many of the signs and symptoms of psychiatric illness. Specifically, single-dose amphetamine treatment in humans reproduces some of the core symptoms of mania: increased energy, euphoria, hyperactivity, decreased need for sleep and psychomotor agitation (14, 15). Chronic use, as seen in amphetamine or cocaine dependence, typically involves an escalation in dose followed by binges. This pattern of use frequently leads to the psychotic symptoms of hallucinations and delusions that can be indistinguishable from those of psychotic mania or the positive symptoms of schizophrenia (16) (Fig 1). This close similarity in clinical presentation suggests that common pathophysiological mechanisms may operate in both amphetamine psychosis and endogenous psychoses.

Numerous lines of evidence argue that this common pathophysiology may involve a disturbance in dopamine neurotransmission. The primary action of amphetamine is to increase synaptic dopamine by stimulating release and blocking reuptake. Axonal projections from dopaminergic cell bodies in the ventral tegmental area to the nucleus accumbens, olfactory tubercle, frontal cortex (prefrontal, cingulate, and entorhinal regions), and amygdaloid nuclei play an important role in regulating motivation and emotion. Furthermore, this mesocorticolimbic dopaminergic system has been implicated in drug addiction and the pathogenesis of mania, depression, and schizophrenia (17, 18). This connection between mood and dopamine is particularly apparent in regard to reward seeking and locomotor activity. Mania is associated with increased reward seeking, a behaviour associated with dopamine release, while depression is associated with anhedonia, a mental state that may result from hypodopaminergia. The large increase in locomotor behaviour of laboratory animals, which is produced by a single injection of amphetamine, has been proposed to model motor hyperactivity observed

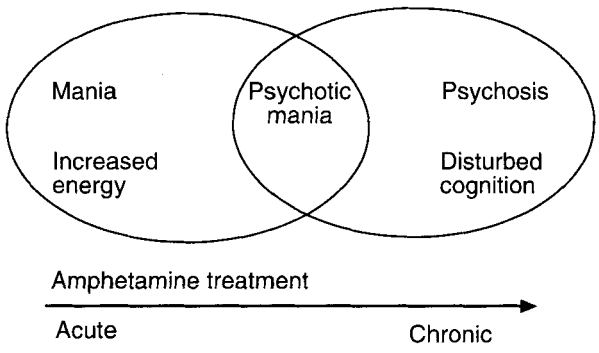

Figure 1. Amphetamine treatment models symptoms of mania and psychosis.

in manic patients. A variety of paradigms of amphetamine treatment in rats have also been used as models of endogenous psychosis. The behavioural sensitization that follows chronic administration of a fixed dose has been a widely used model and likely does reproduce some aspects of human amphetamine abuse (19, 20). More recently, a distinct behavioural profile was identified that emerges from a schedule of escalating doses followed by binges, and that is designed to mimic the human abuse patterns that commonly lead to psychotic symptoms (21).

Animal species

The question of animal species chosen for experiments is an important one. While extensive behavioural pharmacology studies in this area have been reported to date in rats (22, 23), which have somewhat bigger brains, the equivalent studies in mice have been less extensive, as have the analyses of the molecular and histochemical underpinnings. The distinction is important as behaviour, response to drugs and metabolism of drugs are all different in these two rodent species. However, that gap is beginning to be covered. The amenability of mouse to genetic manipulation, and the imminent availability of the complete mouse genome sequence will greatly extend the range of experimental questions that can be addressed in mouse models as compared to rat models.

Gene expression studies

Animal models

Numerous studies of the expression of individual candidate genes have been conducted for several of the above animal models (24). Although these data have provided insights into aspects of likely disease mechanisms, they are subject to a number of limitations. First is the relevance of the animal model to bipolar disorder. Second is the choice of candidate gene to be examined. Third is the difficulty in distinguishing changes that are central to the mechanism of the disease process from those that reflect secondary epiphenomena. By enabling the simultaneous examination of nearly all genes, gene profiling dramatically enhances this approach in several ways. It is comprehensive and thereby facilitates the discovery of unexpected novel gene response. It also permits examination of patterns of gene changes. This power was demonstrated in a study that we have recently conducted of 8000 genes in response to a single dose of methamphetamine in an animal model of mania (4). As described below, several novel and unexpected gene changes were identified that stimulated new directions of inquiry. As this technology is very new, only limited work has been conducted. However, our study suggests that many new discoveries and research directions for bipolar disorder will soon be coming from such approaches.

Post-mortem brain

A number of studies of gene expression in post-mortem brain of bipolar subjects have been conducted to date for specific candidate genes by using several technologies. *In situ* hybridization and immunohistochemistry have the advantage of permitting fine tissue localization of the mRNA and proteins, although their results are more difficult to quantify. Northern blots and Western blots are biochemical methods for quantifying specific RNA, respectively protein, levels from tissue samples. The studies in bipolar disorder where these classic techniques have been employed are somewhat limited, not least by the limited availability of human post-mortem tissue samples. Their main disadvantage is that one has to have a prior hypothesis or an educated guess driving this one-candidate-at-a-time approach.

The mRNAs for complexin I and II, synaptic proteins preferentially expressed by inhibitory and excitatory hippocampal neurones, respectively, were found to be reduced in human post-mortem brain samples in both bipolar disorder and schizophrenia (25). The mRNA and protein levels for GAP-43, a protein involved in the establishment and reorganization of synaptic connections, were found to be decreased in the prefrontal cortex in post-mortem brain samples of depressed patients who committed suicide (26). The mRNA levels of neuropeptide Y were also found to be reduced in the prefrontal cortex of post-mortem brain samples of patients with bipolar disorder, but not major depression or schizophrenia (27). Western blot analysis has also revealed higher levels of the neural cell adhesion molecule (N-CAM) in the hippocampus of bipolar but not schizophrenic patients (28). N-CAM is a cell surface recognition molecule involved in cellular migration, synaptic plasticity and central nervous system development.

Gene expression profiling studies of post-mortem brain have a similar set of advantages and disadvantages as those described above for animal models. In contrast to animal models, the relevance to illness is not an issue; however, post-mortem studies can instead suffer from problems related to certainty of diagnosis and degradation of mRNA. To date, no gene profiling studies of post-mortem brain in bipolar disorder have been reported. However, a recent study of schizophrenia provides a glimpse of the kind of data that future studies may provide. Mirnics and co-workers examined prefrontal cortex in post-mortem brain from subjects with schizophrenia by using microarray analysis (5). They reported that changes in

gene expression were seen primarily in genes related to presynaptic function. Hence, these results support further study of genes based on their subcellular localization and function in the neurone. An important issue for such studies is how to best utilize such information in order to elucidate fundamental mechanisms of illness. This is especially true for genes whose function is unknown and that were discovered in the absence of an a priori hypothesis.

Convergent functional genomics

A combination of complementary strategies

Towards addressing this issue, we have recently proposed an approach that we have termed *convergent functional genomics* that utilizes information from gene profiling studies in order to accelerate the identification of candidate disease susceptibility genes (4). In essence, one would intersect the findings of gene expression studies in animal models with linkage peaks from human familial studies, resulting in a limited number of high-probability candidate genes (Fig 2). Both gene profiling methods and positional cloning share the strength of being comprehensive genomic methods with great potential for discovery of new genetic effects. We argue that their strengths and shortcomings are complementary (Fig 3), and that combining them results in a much greater efficiency in identifying important genes.

The process works by identifying genes whose expression is altered in either an animal model or in post-mortem brain. The chromosomal map position of these genes, or their human homologues in the case of animal models, are then compared with reported linkage peaks for the psychiatric illness under study. Genes whose expression changes in an animal model or in post-mortem brain and map to known linkage peaks are more likely to be susceptibility genes. In this way, functional genomic studies are able to point to a limited number of high-probability candidates within linkage peaks and, thereby, prioritize a smaller number of genes for more detailed and labourious mutation screening. Similarly, of the large number of genes whose expression is altered in a gene profiling study, those that map to linkage hotspots may be more likely to be of primary mechanistic relevance. We have recently reported results of the application of this approach to methamphetamine administration as an animal model of mania. These results provide a proof of principle and suggest that convergent functional genomics may be of use not only in psychiatric disorders, but also other complex genetic traits (4).

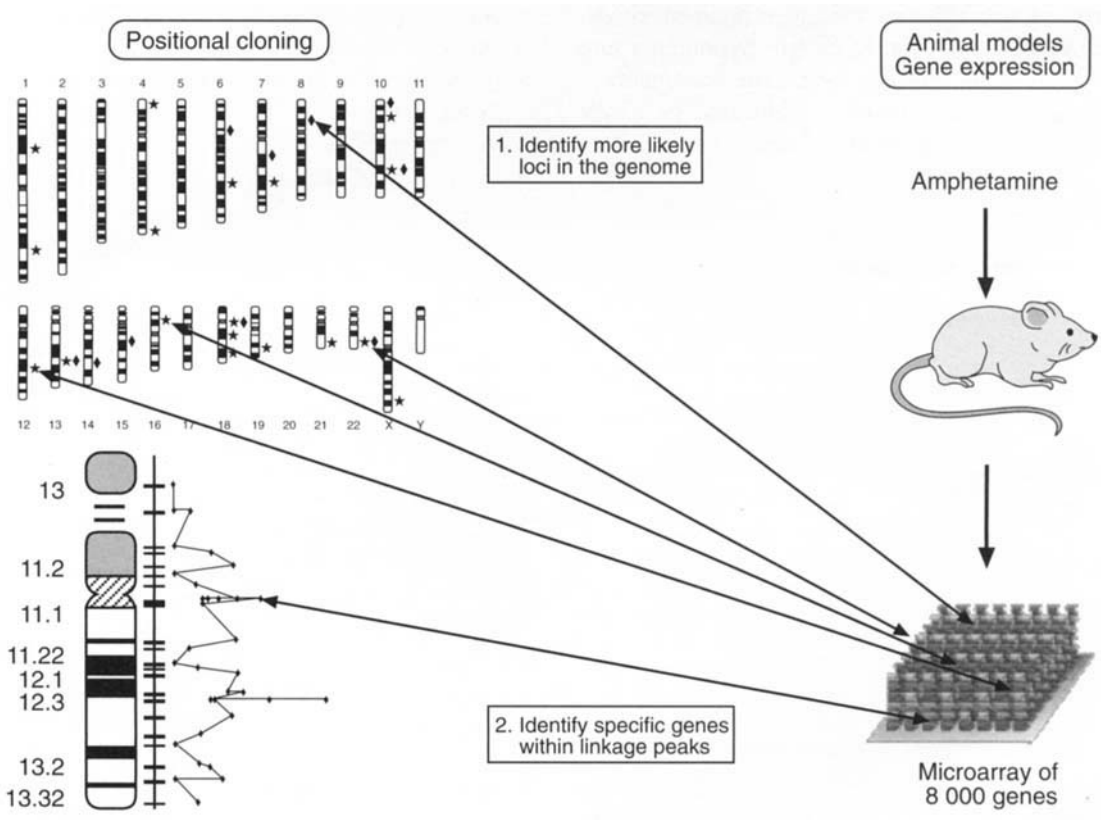

Figure 2. Convergent functional genomics. Genes whose expression is changed in an animal model and map to chromosomal loci identified by linkage are high-probability candidates.

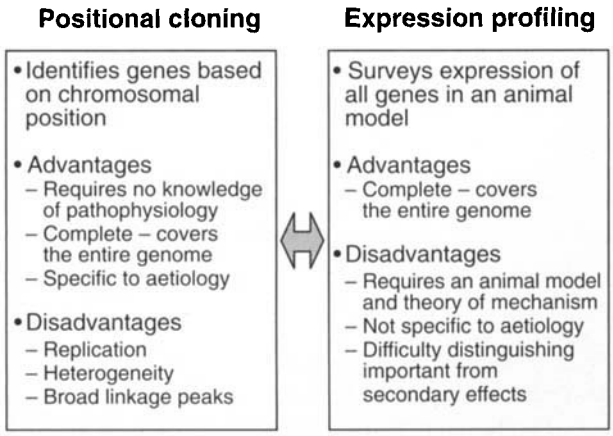

Figure 3. Complementary advantages of positional cloning and expression profiling.

Animal model and design

Rats were administered a single dose of methamphetamine (4.0 mg/kg) as an animal model of mania. Twenty-four hours later the brains were harvested, and specific brain regions (prefrontal cortex-PFC, amygdala-AMY) were dissected out and analysed for gene expression patterns by using microarray technology. The data were then cross-matched against known loci identified by human genetic linkage studies.

In terms of amphetamine treatment regimen, single-dose treatments mimic fairly closely hypomania and mania, whereas in chronic long-term treatments a more complicated picture with additional psychotic features may emerge, as discussed above (Fig 1).

We believe the choice of the timepoint for harvesting to be of critical importance in influencing the results obtained. Harvesting the brain 1 h after the (last) amphetamine dose, as has been the practice in the field, and looking at gene expression, may reveal a very high number of changed transcripts, many of them related to a less specific early-response. Harvesting 24 h later, however, offers a window on gene expression where a lower, more manageable, number of transcripts in general are changed. Furthermore, at 24 h after the first amphetamine treatment, the rat would already manifest an altered behavioural response to another challenge. As it is these lasting changes that are more likely to be related to mania and psychosis, gene changes at this later time point may be more specific and relevant. One obviously needs to perform also time-course experiments to trace the temporal expression profile of the candidate genes identified, which may permit a better mechanistic understanding of their involvement in pathophysiological processes.

Convergent functional genomic candidate genes for bipolar disorder

We employed an oligonucleotide-based method (Affymetrix U34A chip) that measures 7000 cDNAs and 1000 ESTs (expressed sequence tags). An increase or decrease of twofold in each of the two independent experiments was chosen as an empirical cut-off for changes in gene expression. Genes identified by this approach are summarized in Table 1 and Figure 4.

Several genes were striking in the convergence of their degree of induction, known physiological role

Table 1. Genes induced in a methamphetamine model of mania.

Gene symbol	Description	Fold induction	Human chromosomal location	Linkage region*
Prefrontal cortex				
GRK3	G protein-coupled receptor kinase 3	14.2	22q11	B
DBP	D-box binding protein	7.0	19q13.3	B
FDFT1	Farnesyl-diphosphate farnesyltransferase	2.9	8p23.1-p22	S
MALS-1	Vertebrate LIN7 homolog 1	2.9	12q21.3	B
Amygdala				
NDUFS8	NADH –coenzyme Q reductase	20.8	11q13	
SULT1A1	Sulfotransferase 1A1	4.3	16p12.1-p11.2	B
POLR2F	RNA polymerase II polypeptide F	3.9	22q13.1	B, S
FCGRT	IgG Fc receptor transporter alpha	3.2	19q13.3	B
IGF1	Insulin-like growth factor 1	3.0	12q22-q24.1	B
HSPB1	Heat shock protein 27	2.8	7q22.1	
NTRK3	Neurotrophin receptor 3	2.7	15q25	
ADORA3	Adenosine receptor A3	2.7	1p21-p13	
FEZ2	Fasciculation and elongation protein zeta 2 (zygin II)	2.3	2p22	

*Convergent with a linkage peak for either bipolar disorder (B), or schizophrenia (S). (Adapted from (4) with permission.)

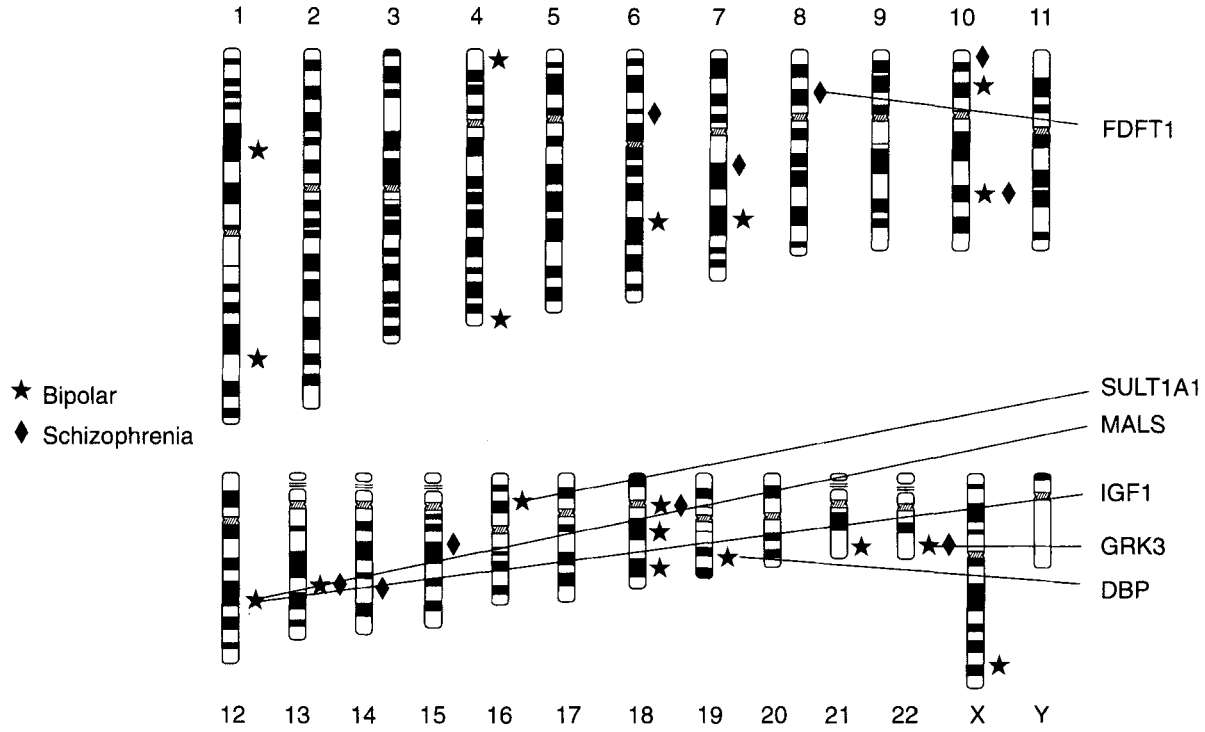

Figure 4. Convergent functional genomic mapping of genes induced in a methamphetamine model of mania to linkage hotspots for bipolar disorder. See Table 1 for definitions of the abbreviations.

and map position. Of these the most impressive was G protein receptor kinase 3 (GRK3). GRK3 underwent an average 14-fold induction and maps to within 20 kb of a linkage peak for bipolar disorder identified in several studies (29–31). GRK3 mediates the homologous desensitization of a variety G protein-coupled receptors, including D1 dopamine receptors. This suggests the attractive hypothesis that a defect in GRK3 impairs the desensitization of D1 receptors in response to increased dopamine neurotransmission and, thereby, results in an effective supersensitivity to dopamine. Such a neural mechanism for mania and psychosis has been suspected for several decades. At this point, these results are only suggestive and require the identification of a functional mutation in the gene and its association to illness in an independent sample.

Several other identified genes are also intriguing candidates worthy of further detailed study. These include the clock gene, DBP; a gene involved in cholesterol biosynthesis, FDFT1; insulin-like growth factor 1, IGF1, which has been shown to regulate development and function of dopamine neurons; SULT1A1, which inactivates dopamine by sulfation; and MALS-1, a synaptic protein involved in glutaminergic neurotransmission.

Clearly, given the number of genes induced and the number of linkage peaks, it is possible that some of these genes converge on linkage peaks by chance. Furthermore, it is possible that the mutation in the susceptibility gene in any given linkage peak may not undergo a change in level of expression in either ill individuals or in an animal model. Such a gene would not be detected by this approach. Rather, the advantage of this approach is its ability to focus the next stage of mutation screening on a smaller number of higher probability candidates.

Grouping candidate genes in classes: psychogenes and psychosis suppressor genes

By analogy to cancer biology, we proposed that genes involved in psychiatric disorders can be viewed as falling into two prototypical categories. Genes whose activity promotes processes that lead to psychiatric disorders could be called psychogenes, by analogy to oncogenes. Conversely, genes whose activity suppresses processes that lead to psychiatric disorders could be called psychosis suppressor genes, by analogy to tumour suppressor genes (Table 2). Though this breakdown is simplistic, it does have a heuristic value in considering the roles of these putative disease genes in pathophysiology and as targets for therapeutic intervention.

Table 2. Candidate psychogenes and psychosis suppressor genes.

Psychogenes	Psychosis suppressor genes
DBP	GRK3
FDFT1	SULT1A1
MALS-1	
IGF1	

See Table 1 for definitions of the abbreviations. (Adapted from (4) with permission.)

Future directions

Regulomics

The limited number of genes in the human genome, the large quantities of nonprotein coding DNA, the likely mutations in those regions and their impact on gene expression and complex regulatory networks may be the emerging theme in psychiatric genetics, and genetics in general, in the years to come. While current areas of attention are the Human Genome Project, Genomics and Proteomics, we would submit that a Human Promoter Project and Regulomics might be next in terms of large-scale projects with high impact and the logical future direction for functional genomics.

Dissecting functional cascades in mouse knock-out models

In the meantime, the candidate genes identified by approaches, such as convergent functional genomics, and validated by the identification of functional polymorphisms, need to be further understood and dissected in terms of their molecular cell biology.

Mouse models generated through directed targeted gene alterations (reverse genetics), or generated serendipitously through large-scale mutagenesis (forward genetics), will provide the necessary model systems for basic science as well as pharmacological and drug development studies. Mice carrying multiple mutations through breeding of different individual mutants may become the norm and mimic more closely the complex pathophysiology of these disorders as present in the human disease counterpart.

Mouse knock-out models for some of the candidate genes that we have identified have actually been generated independently by other groups (GRK3, DBP and IGF1), and should provide fertile ground for future research.

Endophenotypes as a rate-limiting step in further progress

It is likely that the complex problems of understanding psychiatric disorders will be solved by a concerted, multifaceted approach, involving molecular genetics, brain imaging and improved instruments for phenotype assessment (32). The problem of endophenotypes in psychiatry is an especially acute one, because of a combination of inherent complexity, subjectivity and limited quantifiability proper to this field. With the rapid advances in genomics and imaging, this may in fact become the rate-limiting step for future rapid progress. It is hoped that the constant interplay between clinical research, neurophysiology, imaging and molecular genetics will lead us past this potential roadblock (33). Awareness and concerted efforts are key to the 'recovery' of psychiatry from being a 'soft' field.

References

1. **Goodwin FK, Jameson KR.** *Manic-depressive illness.* New York: Oxford University Press; 1990.
2. **Kelsoe JR.** The genetics of mood disorders. In: Kaplan HK Saddock BJ, eds. *Comprehensive textbook of psychiatry,* 7th edn. Baltimore: Williams and Wilkins; 2000; 1308–18.
3. **Terwilliger JD, Shannon WD, Lathrop GM, Nolan JR, Golding LR, Chase GA, et al.** True and false positive peaks in genomewide scans: applications of length-biased sampling to linkage mapping. *Am J Hum Genet* 1997; 61: 430–8.
4. **Niculescu AB, Segal DS, Kuczenski R, Barrett T, Hauger RL, Kelsoe JR.** Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. *Physiol Genomics* 2000; 4: 83–91.
5. **Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P.** Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* 2000; 28: 53–67.
6. **Watson SJ, Meng F, Thompson RC, Akil H.** The "chip" as a specific genetic tool. *Biol Psychiatry* 2000; 48: 1147–56.
7. **Lewohl JM, Dodd PR, Mayfield RD, Harris RA.** Application of DNA microarrays to study human alcoholism. *J Biomed Sci* 2001; 8: 28–36.
8. **El Mallakh RS, Harrison LT, Li R, Changaris DG, Levy RS.** An animal model for mania: preliminary results. *Prog Neuropsychopharmacol Biol Psychiatry* 1995; 19: 955–62.
9. **Li R, El Mallakh RS, Harrison L, Changaris DG, Levy RS.** Lithium prevents ouabain-induced behavioral changes. Toward an animal model for manic depression. *Mol Chem Neuropathol* 1997; 31: 65–72.
10. **Decker S, Grider G, Cobb M, Lix P, Huff MO, El-Mallakh RS, et al.** Open field is more sensitive than automated activity monitor in documenting ouabain-induced hyperlocomotion in the development of an animal model for bipolar illness. *Prog Neuropsychopharmacol Biol Psychiatry* 2000; 24: 455–62.
11. **Rose AM, Mellett BJ, Valdes R Jr, Kleinman JE, Herman MM, Li R, et al.** Alpha 2 isoform of the Na,K-adenosine triphosphatase is reduced in temporal cortex of bipolar

individuals. *Biol Psychiatry* 1998; 44: 892–7.
12. **Gessa GL, Pani L, Fadda P, Fratta W.** Sleep deprivation in the rat: an animal model of mania. *Eur Neuropsychopharmacol* 1995; 5 Suppl: 89–93.
13. **Petty F, Sherman AD.** A pharmacologically pertinent animal model of mania. *J Affect Disord* 1981; 3: 381–7.
14. **Fibiger HC.** The dopamine hypothesis of schizophrenia and mood disorders: contradictions and speculations. In: Willner P, Scheel-Kruger J, eds. *The mesolimbic dopamine system: from motivation to action.* Chichester, UK: John Wiley and Sons; 1991: 615–37.
15. **Jacobs D, Silverstone T.** Dextroamphetamine-induced arousal in human subjects as a model for mania. *Psychol Med* 1986; 16: 323–9.
16. **Angrist B.** Amphetamine psychosis: clinical variations of the syndrome. In: Cho AK, Segal DS, eds. *Amphetamine and its analogues.* San Diego, CA: Academic Press; 1994: 387–414.
17. **Wilner P.** Dopaminergic mechanisms in depression and mania. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: The fourth generation of progress.* New York: Raven Press; 1995: 921–31.
18. **Koob GF, Sanna PP, Bloom FE.** Neuroscience of addiction. *Neuron* 1998; 21: 467–76.
19. **Segal DS, Schuckit MA.** Animal models of stimulant-induced psychosis. In: Creese I, ed. *Stimulants: neurochemical, behavioral and clinical perspectives.* New York: Raven Press; 1983: 131–67.
20. **Robinson TE, Becker JB.** Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res* 1986; 396: 157–98.
21. **Segal DS, Kuczenski R.** An escalating dose "binge" model of amphetamine psychosis: behavioral and neurochemical characteristics. *J Neurosci* 1997; 17: 2551–66.
22. **Janowsky DS, Risch C.** Amphetamine psychosis and psychotic symptoms. *Psychopharmacology (Berl)* 1979; 65: 73–7.
23. **Acquas E, Fibiger HC.** Chronic lithium attenuates dopamine D1-receptor mediated increases in acetylcholine release in rat frontal cortex. *Psychopharmacology (Berl)* 1996; 125: 162–7.
24. **Shilling PD, Kelsoe JR, Kuczenski R, Segal DS.** Differential regional zif268 messenger RNA expression in an escalating dose/binge model of amphetamine-induced psychosis. *Neuroscience* 2000; 96: 83–90.
25. **Eastwood SL, Harrison PJ.** Hippocampal synaptic pathology in schizophrenia, bipolar disorder and major depression: a study of complexin mRNAs. *Mol Psychiatry* 2000; 5: 425–32.
26. **Carter DA.** Temporally defined induction of c-fos in the rat pineal. *Biochem Biophys Res Commun* 1990; 166: 589–94.
27. **Caberlotto L, Hurd YL.** Reduced neuropeptide Y mRNA expression in the prefrontal cortex of subjects with bipolar disorder. *Neuroreport* 1999; 10: 1747–50.
28. **Vawter MP, Hemperly JJ, Hyde TM, Bachus SE, Van der Putten DM, Howard AL, et al.** VASE-containing N-CAM isoforms are increased in the hippocampus in bipolar disorder but not schizophrenia. *Exp Neurol* 1998; 154: 1–11.
29. **Kelsoe JR, Spence MA, Loetscher E, Foguet M, Sadovnick AD, Remick RA, et al.** A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. *Proc Natl Acad Sci U S A* 2001; 98: 585–90.
30. **Edenberg HJ, Foroud T, Conneally PM, Sorbel JJ, Carr K, Crose C, et al.** Initial genomic scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 3, 5, 15, 16, 17, and 22. *Am J Med Genet* 1997; 74: 238–46.
31. **Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G, et al.** A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci U S A* 1999; 96: 5604–9.
32. **Niculescu III AB.** Brainology. *Med Gen Med* 1999; E21.
33. **Niculescu AB, Akiskal HS.** Endophenotypes of dysthymia: evolutionary, clinical and pharmacogenomic considerations. *Mol Psychiatry* (in press)