Identification of molecular mechanisms underlying mood stabilization through genome-wide gene expression profiling

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Although Kraepelin described bipolar disorder (BD) to have a relatively good prognosis (Kraepelin, 1921), more recent studies have clearly shown that this may only represent the situation for a minority of subjects afflicted with this devastating illness; thus, a number of studies and analyses have now shown that for a large percentage of patients, outcome is quite poor, with high rates of relapse, chronicity, lingering residual symptoms, subsyndromes, cognitive and functional impairment, and psychosocial disability (Judd et al., 2002, 2003; Tohen et al., 2003; Zarate et al., 2000). Furthermore, suicide is estimated to be the cause of death in up to ~15% of individuals with mood disorders. In addition to suicide, many other deleterious health-related effects are increasingly being recognized (Kupfer, 2005). The costs associated with disability and premature death represent an economic burden of tens of billions of dollars annually in the United States alone. It is, thus, not altogether surprising that the Global Burden of Disease Study has identified mood disorders among the leading causes of disability worldwide, and as illnesses that are likely to represent an increasingly greater health, societal, and economic problem in the coming years (Simon, 2003).

Increasing evidence suggests that a significant number of patients do not respond adequately to therapy with present existing mood-stabilizing agents, there is, thus, a clear need to develop improved therapeutics for BD. Previous studies were, by and large, designed to detect relative excess or deficiency associated with pathological states; not surprisingly, progress in unravelling the unique neurobiology of this disorder was slow using such strategies in isolation. However, the molecular medicine revolution has brought to bear the power of sophisticated cellular and molecular biological methodologies to tackle many of society’s most devastating illnesses and allowed the study of a variety of human diseases that are caused by abnormalities in cell-to-cell communication; studies of such diseases are offering unique insights into the physiological and pathophysiological functioning of many cellular signalling cascades. It is now clear that the complex behavioural and physiological manifestations of BD are probably mediated by a disruption of a network of interconnected neuronal circuits (Payne et al., 2004), rather than any single neurotransmitter deficiency or excess per se (Coyle and Duman, 2003). In addition, although acute, in-vitro effects of mood stabilizers have previously been identified, their therapeutic effects in the treatment of BD are seen only after chronic administration (Quiroz et al., 2004); thereby precluding simple mechanistic interpretations based on their acute biochemical effects. In view of this analysis, surveys of molecular targets by mood-stabilizing agents like lithium and valproate after chronic treatment may cast new light on the delicate molecular mechanisms for their therapeutic significance in BD (Zhou et al., 2005).

The role of gene expression profiling in psychiatric research

There have been tremendous advances in the last decade in our ability to study an entire transcriptome (all the genes transcribed at one time) and proteome (all the genes translated at one time, as well as their various post-translational modifications); these methodologies are not only providing important new leads in our understanding of the molecular and cellular pathophysiology of severe psychiatric disorders, but also showing insight into ways for developing novel...
treatments. The advent of methodologies such as subtractive hybridization, mRNA differential display (DD), and microarrays has illustrated the importance of hypothesis-generating (as opposed to hypothesis-dependent) techniques, particularly when dealing with disorders whose pathophysiology remains largely unknown. The power of microarray – the newest of the techniques – is self-evident; this approach has largely become the method of choice to interrogate the whole transcriptome. In recent years, microarray studies have been increasingly applied in researches on psychiatry disorders, including schizophrenia (Wong et al., 2004), major depression (Evans et al., 2004) and BD (Ogden et al., 2004), most of the studies have provided hitherto unnoticed genes or/and mechanisms of mental disorders and their treatments. In this issue of the Journal, Chetcuti et al. (2005) report on their findings using the microarray methodology to identify novel targets for the actions of the mood-stabilizing anticonvulsant valproate. Prior to discussing these novel findings, a brief discussion about the strengths and limitations of the microarray methodology is warranted. It is important to note that microarray technology should not necessarily be considered to be superior to, or a replacement of, DD. Rather, each technique has unique advantages and disadvantages. The microarray methodology only allows for the identification of known transcripts (which the array in question comprises of); by contrast, DD allows the detection of entirely novel mRNA transcripts. The reader can refer to the review by Liang (2002) for further comparison.

One key difficulty in the use of microarrays lies in the signal-to-noise ratio. Particularly for transcripts of low abundance, the test–retest reliability of microarray results can be dismal. For this reason, repetition is of obvious importance (Lee et al., 2000). Unfortunately, the high cost of microarrays often forces researchers to forego sufficient replication, thereby jeopardizing the usefulness of their results. Likewise, the commonly used approach of establishing a threshold of what magnitude of expression ratio constitutes a significant change fails to address the issue of deviation and error. As the reliability increases, and the cost decreases, microarray data will hopefully become subject to more statistically rigorous analyses.

In this light, the stringency with which microarray data are analysed becomes a much fuzzier matter, and may be adjusted to address the specific question under investigation. While at first glance one may question the scientific rigour of such an approach, it needs to be emphasized that the microarray methodology is only a screening technique, and the results require much more independent validation (e.g. quantitative RT–PCR, protein levels analysis at relevant brain regions, and specific functional studies). Thus, the cut-off threshold criteria should be determined not only by statistical considerations, but also the stringency and rigour of the experimental paradigm (e.g. identifying common targets of structurally highly dissimilar drugs of the same category, such as lithium and valproate, to enhance specificity), and the ability/willingness to subsequently validate positive results with independent methodologies.

Besides fold-difference comparisons and statistical tests (such as $t$ test) for consistency, clustering is a fairly common technique in which transcripts are associated based on their co-regulation across a number of samples. ‘Fingerprinting’ refers to the association of a general pattern of microarray results with a particular variable. For example, this technique has already begun to show usefulness in oncology, where patterns in microarray data from biopsies may help predict treatment response and outcome. Likewise, ex-vivo gene expression data (i.e. data from microarray analysis using biopsy samples) of psychiatric patients may one day predict optimal treatment strategies, risk of relapse, etc.

Despite this tremendous progress, the current transcriptomics methodologies still have major limitations, foremost amongst these are the inability to distinguish between splice variants and only high-to-medium abundance transcripts can be profiled with relative accuracy. Furthermore, there are additional uncertainties inherent in the study of post-mortem brain tissue. Variables such as post-mortem delay (Ryan et al., 2004), ante- or peri-mortem factors like hypoxia (Harrison et al., 1995) can affect mRNA integrity. Finally, for gene expression changes to be related to functional neuronal changes, expression at the protein level should be examined (the genome is the script, and the proteins are the actors). For these reasons, the use of advanced proteomics methodologies in conjunction with transcriptomics is most likely to yield critical information about psychiatric disorders.

In Chetcuti and colleagues’ report in this issue, 11 genes were identified by fold changes and four (Zinc finger protein of the cerebellum 1, SCM-like with four mbt domains 2, Structural maintenance of chromosomes 4-like 1, and Prostate apoptosis response-4) of them were confirmed by quantitative PCR. Notably, these genes can be classified as playing critical roles in neuronal survival, neuronal differentiation and vesicle biogenesis. The identification of genes regulating cellular plasticity and resilience as
targets for mood stabilizers is particularly noteworthy since—although BD has traditionally been conceptualized as a neurochemical disorder—there is now evidence from a variety of sources demonstrating regional reductions in CNS volume, as well as reductions in the numbers and/or sizes of glia and neurons in discrete brain areas (Manji et al., 2003; Rajkowska, 2000), suggesting that severe BDs are associated with impairments of structural plasticity and cellular resilience. It is, thus, worth mentioning that recent preclinical studies—including the present one reported in this issue of the Journal—have shown that critical molecules in neurotrophic signalling cascades are long-term targets for mood-stabilizing agents. Consistent with these biochemical effects, mood-stabilizing agents have been demonstrated to exert robust neuroprotective and neurotrophic effects in a variety of preclinical paradigms (Yuan et al., 2004). Thus, optimal treatment may only be attained by providing both trophic and neurochemical support; the trophic support would be envisioned as enhancing and maintaining normal synaptic connectivity, thereby allowing the chemical signal to reinstate the optimal functioning of critical circuits necessary for normal affective functioning (Du et al., 2004). There are a number of pharmacological plasticity enhancing strategies which may be of considerable utility in the treatment of mood disorders (Quiroz et al., 2004; Zarate et al., 2005). This research holds much promise for the development of novel therapeutics for the treatment of severe, refractory mood disorders. It is anticipated that—in the coming years—the concerted use of genomic and proteomic strategies to refine complex psychiatric diseases into mechanism-based subcategories may ultimately allow for the matching of particular target-based therapies to particular markers in subgroups of patients.

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Statement of Interest

None.

References


