Review

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Biomarkers in bipolar disorder: A positional paper from the International Society for Bipolar Disorders Biomarkers Task Force

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Abstract

Although the etiology of bipolar disorder remains uncertain, multiple studies examining neuroimaging, peripheral markers and genetics have provided important insights into the pathophysiologic processes underlying bipolar disorder. Neuroimaging studies have consistently demonstrated loss of gray matter, as well as altered activation of subcortical, anterior temporal and ventral prefrontal regions in response to emotional stimuli in bipolar disorder. Genetics studies have identified several potential candidate genes associated with increased risk for developing bipolar disorder that involve circadian rhythm, neuronal development and calcium metabolism. Notably, several groups have found decreased levels of neurotrophic factors and increased pro-inflammatory cytokines and oxidative stress markers. Together these findings provide the background for the identification of potential biomarkers for vulnerability, disease expression and to help understand the course of illness and treatment response. In other areas of medicine, validated biomarkers now inform clinical decision-making. Although the findings reviewed herein hold promise, further research involving large collaborative studies is needed to validate these potential biomarkers prior to employing them for clinical purposes. Therefore, in this positional paper from the ISBD-BIONET (biomarkers network from the International Society for Bipolar Disorders), we will discuss our view of biomarkers for these three areas: neuroimaging, peripheral measurements and genetics; and conclude the paper with our position for the next steps in the search for biomarkers for bipolar disorder.

Keywords

Biomarkers, bipolar disorder, inflammation, neutrophins, oxidative stress

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Introduction

The current diagnostic criteria for psychiatric diagnoses are based purely on self-report and behavioral observation and lack substantial biological validation. This contrasts sharply with several other areas of medicine where biological tests, based on validated biomarkers, aid in diagnostic and treatment decisions. Biological markers, or biomarkers, are measurements that quantify biological processes, a disease state, or response to treatment. A biomarker will be clinically useful only if it is accurate, reproducible and acceptable to the patient, and has ease of interpretation, adequate sensitivity and specificity, and association with treatment response (Vasan, 2006). The discovery of a biomarker usually encompasses two complimentary approaches: a deductive, knowledge-based method followed by an unbiased inductive strategy. The deductive method is associated with a previous understanding of an underlying pathophysiological process, whereas the inductive strategy involves processes such as the measurement of a large number of potential molecules (e.g. array). Critical initial steps should be taken in the development of a biomarker, which start with the identification (construct validity, Step 1) and validation (criterion validity, Step 2) of the assays or similar means of measurement and quantification, which may occur via the use of cross-sectional studies with small sample sizes. Steps 3 and 4 include the distribution of the biomarker in cases and controls (predictive validity) and the ability to predict disease longitudinally (efficacy of strategy). Finally, when a biomarker displays adequate sensitivity, specificity and predictive value in the validation phase, its effect on diagnostic or prognostic outcomes or its cost-effectiveness can be determined in the context of a randomized controlled trial (RCT) (Step 5). The lack of RCTs in psychiatric research, therefore, reflects the newness of the development of biomarkers in mental illness (Van Lieshout and Szatmari, 2009). It is important to keep in mind, however, that while the methodological rigor increases from cross-sectional and case–control studies to longitudinal cohorts with RCTs, in some circumstances cross-sectional studies can provide highly valuable information about the accuracy of a test (Schunemann et al., 2008). There is an extensive literature on the concept, development and validation of biomarkers which is well reviewed elsewhere (Frank and Hargreaves, 2003).

There are compelling reasons to consider in the development of a strategy for biomarker discovery, validation and application in bipolar disorder (BD). First, biomarkers could potentially improve diagnostic accuracy when added to clinical interviews and observation. Second, there is a current emphasis in clinical research in BD on prevention strategies at the primary (i.e. new onset), secondary (early symptom onset) and tertiary (relapse/recurrent or progressive disability) levels in addition to staging of the illness (i.e. course of illness, burden of illness, and the need for multiple drug treatments). This approach requires refinement of our current diagnostic method such as the development of biomarkers. As there is an increasing shift towards personalized medicine, improved tools for risk assessment and treatment selection are needed (Pasco et al., 2010). In BD, biomarkers may be useful tools in detecting disease activity associated with various mood states (a 'state marker') or identifying specific features that are observed in the long-term course of the illness (a 'trait marker'). 'State markers' could help in differentiating between opposite poles (mania vs depression) or may be only useful when measured during a specific mood episode.

The present paper is not an exhaustive account of recent findings in biological research in BD. Instead, evidence reviewed in this paper comprises a selective review of the literature, with the goal of reconciling the large body of biological research in BD into a select handful of potential biomarkers that in our view are the most promising. We focused on evidence supporting the potential role of biomarkers in BD, and we highlighted promising new avenues indentified by the International Society for Bipolar Disorders (ISBD) Biomarkers Network Task Force. The next sections will describe some potential candidates for biomarkers in three main areas: neuroimaging, peripheral biomarkers and genetics (Figure 1). With the increasing focus on early identification and intervention, each section will include specific reference to potential biomarkers in pediatric BD. The reader is referred to previous articles that reviewed the validity, diagnosis and differential diagnosis of pediatric BD (Goldstein, 2012; Youngstrom et al., 2008).

Neuroimaging

Structural imaging

The most common structural abnormalities reported in BD are increased lateral ventricular volumes and higher rates of white matter hyperintensities (Hallahan et al., 2011; Kempton et al., 2008). However, these findings are relatively non-specific as they occur in a number of pathologies and are even identified in normal aging (Gootjes et al., 2004). While there is now substantial evidence that lithium increases the gray matter volume of prefrontal cortex (Moore et al., 2009), amygdala (Foland et al., 2008; Savitz et al., 2010) and hippocampus (Bearden et al., 2008; Yucel et al., 2008), the impact of increased gray matter volume in these brain regions on the long-term course of BD is still unknown. These findings of increased gray matter volume after lithium treatment seem to be independent of mood states; some studies were conducted in bipolar subjects in a depressive episode (Moore et al., 2009; Savitz et al., 2010) whereas others included bipolar subjects in various mood states (Bearden et al., 2008; Foland et al., 2008; Yucel et al., 2008). Despite these encouraging initial findings, whether or not increase in gray matter is a marker of clinical

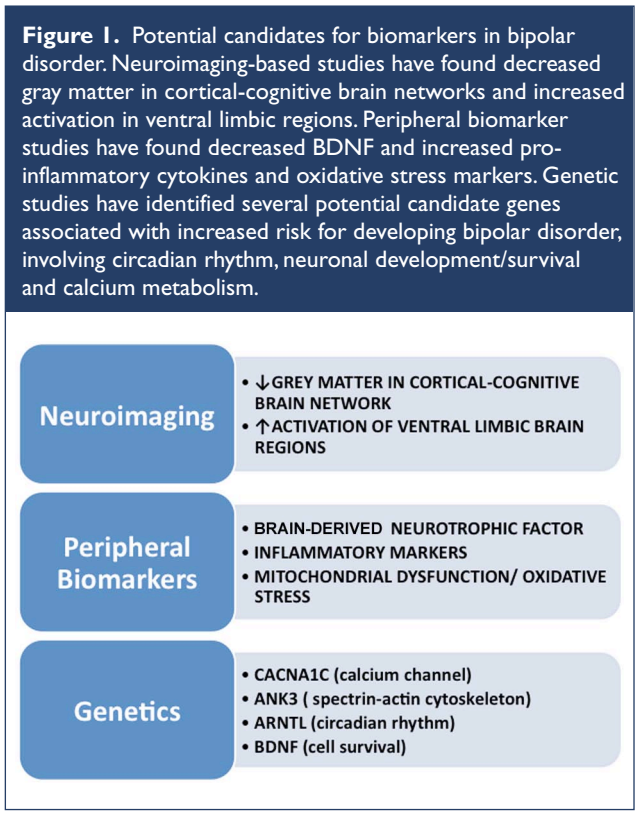

Figure 1. Potential candidates for biomarkers in bipolar disorder. Neuroimaging-based studies have found decreased gray matter in cortical-cognitive brain networks and increased activation in ventral limbic regions. Peripheral biomarker studies have found decreased BDNF and increased pro-inflammatory cytokines and oxidative stress markers. Genetic studies have identified several potential candidate genes associated with increased risk for developing bipolar disorder, involving circadian rhythm, neuronal development/survival and calcium metabolism.

response, it remains to be established. Neuroimaging studies of pediatric BD converge with adult studies in implicating fronto-limbic structures (Caetano et al., 2005). However, the most replicated finding in youth, not consistently observed in adults, is that of smaller amygdala size versus healthy controls (Pfeifer et al., 2008). It is conceivable that differences in amygdala volume between pediatric and adult BD may be due to medication effects, developmental compensatory mechanisms, or the effects of substance use. Alternatively, the heterogeneity of findings among adults may be due to combining adults with pediatric-onset and adult-onset BD in the same analyses.

Diffusion tensor imaging (DTI) may provide a better assessment of white matter integrity. Specifically, microstructural abnormalities of the myelinated tracts of the prefrontal cortex have been observed in BD (Adler et al., 2004, 2006; Benedetti et al., 2011; Beyer et al., 2005; Haznedar et al., 2005; Houenou et al., 2007; Regenold et al., 2006). These tracts provide connectivity between subcortical limbic and striatal regions and the prefrontal cortices, and are therefore integral to the functional neurocircuitry that appears to be disrupted in BD. Recent studies suggesting that decreased fronto-temporal white matter fractional anisotropy may be able to differentiate between BD and major depressive disorder deserves further investigation and replication (Benedetti et al., 2011; Versace et al., 2010). Preliminary findings suggest that white matter pathology may also be implicated in pediatric BD (Adler et al., 2006).

Functional imaging

Functional neuroimaging studies might identify patterns of brain activation or connectivity that could potentially predict subsequent conversion to BD in individuals at high risk (i.e. unaffected first-degree relatives), aid in the differential diagnosis of mood disorders (i.e. unipolar vs bipolar depression) and/or guide treatment selection. Current models that propose a neurobiological basis for emotional dysregulation suggest that BD is associated with abnormalities within fronto-limbic-subcortical structures (Phillips et al., 2008; Savitz and Drevets, 2009; Strakowski et al., 2005). Such abnormalities are often described as increased 'bottom-up' and/or decreased 'top-down' regulation of mood. Others have proposed an overactive (emotional) 'ventral system' including amygdala, ventral striatum, ventral anterior cingulated cortex (ACC), ventral prefrontal cortex (PFC) and insula, and a hypoactive (cognitive) 'dorsal system' including hippocampus, dorsal ACC and dorsal PFC (Phillips et al., 2003).

Two independent fMRI studies that investigated patterns of brain activation in individuals at high risk to develop BD during a working memory task (N-Back) found that individuals at risk exhibit greater activation in the frontal polar cortex (BA 10) as compared to controls, while a trend in the same direction was observed in BD subjects (Drapier et al., 2008; Thermenos et al., 2010). These studies suggest that hyperactivation in this particular brain area with increased memory load may be a marker of increased risk for development of BD (or endophenotype). Further, although increased amygdala activation during presentation of emotional faces does not seem to differentiate BD from other major psychiatric conditions, a recent study suggested that the degree of amygdala activation in response to neutral and mildly sad faces may differentiate bipolar versus unipolar depression (Almeida et al., 2010). Such research emphasizes network disruptions, with overactivity within regions involved in emotion regulation and impaired top-down regulation of ventral structures by regions important in cognitive control. Variability in paradigms, acquisition approaches (e.g. 1.5 T vs 3 T; use of different magnetic resonance imaging (MRI) scanners), analytic techniques (e.g. whole-brain vs regions-of-interest) and phenotypic characterization (e.g. BD-I vs BD-II vs BD-NOS (not otherwise specified); lack of control of length of illness; number of previous mood episodes, etc.) are likely associated with some of the inconsistencies.

Recent meta-analyses of fMRI studies that have contrasted bipolar subjects (all mood states) with healthy controls during emotional tasks, have more consistently found increased activation in the parahippocampal gyrus, amygdala, basal ganglia thalamus and middle frontal gyrus (BA 10), but decreased activation in the inferior frontal gyrus (IFG; or ventrolateral PFC, BA 47), precuneus, middle frontal gyrus (BA 9), thalamus and cerebellum (Chen et al.,

2011; Houenou et al., 2011). During cognitive control tasks, individuals with BD display a decreased activation in the IFG, lingual gyrus and putamen (Chen et al., 2011; Pompei et al., 2011). In studies comparing BD subjects at different mood states during emotional tasks, the most consistent findings have been a decreased activation in the IFG in manic patients versus controls and an increased activation in the parahippocampal gyrus in euthymic patients versus controls (Chen et al., 2011; Houenou et al., 2011; Strakowski et al., 2011).

There is evidence of functional neuroanatomical differences among children and adolescents with BD versus controls, subjects with attention-deficit hyperactivity disorder (ADHD), and subjects with chronic non-episodic severe irritability including activation in response to images of faces of various valences (Brotman et al., 2008; Chang et al., 2004; Dickstein et al., 2007; Pavuluri et al., 2007) and during motor inhibition (Leibenluft et al., 2007). In our view, the potential use of fMRI in the prediction of treatment response in BD has been a largely neglected area of research. This may be related to the significant practical difficulties in the recruitment of unmedicated subjects, and the multiple different combinations of treatments that are commonly used in BD presents challenges to recruiting adequately large samples. In summary, functional imaging studies support the proposed models suggesting overactivation of limbic structures and underactivation of top-down cognitive control in BD. To date, the most consistent finding suggests that individuals with BD fail to activate the IFG during tasks that involve both emotional and non-emotional processing, especially during manic phases. Given that mania is the clinical hallmark of BD, IFG activity emerges as a potential biomarker. Future studies might use the failure to activate IFG as a region of interest (ROI) a priori, and determine whether these differences are found in individuals with genetic risk to develop BD, are specific to BD (e.g. vs unipolar depression or schizophrenia), or might predict treatment response. Finally, at present, there are no longitudinal studies using the same fMRI paradigm at different time points in the course of illness and, in particular, to evaluate the various mood states and prediction of treatment response. Although there are clearly practical considerations with using this finding as a potential biomarker, such as cost and availability of expertise in fMRI, further studies with this approach are highly encouraged.

Spectroscopy

Proton MR spectroscopy (^{1}H-MRS) is an in vivo, non-invasive brain imaging technique that can detect alterations in brain biochemistry in the presence of normal anatomy. As reviewed elsewhere (Stork and Renshaw, 2005), resonances in the ^{1}H-MRS spectrum can be reliably quantified for several metabolites with brain concentrations in the millimolar range, including: *N*-acetyl-aspartate (NAA), a marker of neuronal viability; the excitatory amino acid glutamate (Glu); glutamine (Gln), the glial cell reservoir storage form of glutamate; and myo-inositol (mI), a component of the cellular phosphoinositol-cycle second messenger system. All of these neurometabolites have been studied in BD.

While there are very few published systematic studies using ^{1}H-MRS in populations at risk to develop BD (Gallelli et al., 2005; Hajek et al., 2008), there is some evidence that glutamate levels may differ between subjects with bipolar and unipolar depression. Previous ^{1}H-MRS studies of depressed patients reported a variety of findings in both ACC and other prefrontal (dorsolateral, dorsal anterolateral, dorsomedial, medial, ventromedial) cortical regions. The most consistent results across several studies that have been recently reviewed elsewhere (Yuksel and Ongur, 2010) are increased (Dager et al., 2004; Frye et al., 2007; Ongur et al., 2008) or normal (Port et al., 2008) ACC glutamate in bipolar depression. In contrast, in was found that ACC glutamate in unipolar depressed patients was either decreased (Auer et al., 2000) or not different than healthy controls (Price et al., 2009; Walter et al., 2009) and that levels may increase after treatment (Pfleiderer et al., 2003). Studies using ^{1}H-MRS in other brain areas are less conclusive than those of ACC.

^{1}H-MRS also shows promise in finding biomarkers of treatment response based on the studies published to date. For example, treatment with either lithium or lamotrigine has been shown to increase gray matter volume and/or NAA levels in bipolar depression (Frye et al., 2007; Moore et al., 2000, 2009). Furthermore, changes in glutamate or glutamine have been associated with treatment response in depressed BD subjects treated with riluzole and lamotrigine (Brennan et al., 2010; Frye et al., 2007). Specifically, treatment with riluzole was associated with a rapid decrease in the glutamine/glutamate ratio and with a significant decrease in Hamilton depression rating scale scores (Brennan et al., 2010). Remission with lamotrigine treatment was associated with lower post-treatment glutamine levels as compared to non-remission (Frye et al., 2007). There are also preliminary data using this approach in adolescents with bipolar depression, with reports of variable region-specific neurochemical changes following lithium treatment (Patel et al., 2006, 2008). Further studies are encouraged to evaluate the response prediction of these biomarkers.

Peripheral biomarkers for BD

There has been a long history of interest in peripheral biomarkers in psychiatric illnesses. The particular advantage of this approach is that such tests would be widely available, of low cost and amenable to large-scale studies, including those with longitudinal follow-up. As early as the 1970s there was much interest in peripheral biomarkers in patients with mood disorders, including BD (Maj et al., 1984;

Platman and Fieve, 1968), and much of this centered around the hypothalamic-pituitary-adrenal (HPA) axis (i.e. cortisol and dexamethasone suppression test) and monoaminergic neurotransmitters, such as measuring metabolites in serum and urine and later platelet ^{3}H-imipramine binding. Several reports have demonstrated that patients in both depressive and manic episodes present increased levels of serum, urinary and salivary cortisol (de Kloet et al., 2005; Kutcher and Sokolov, 1995; Pariante, 2004). Dexamethasone challenge was shown to not suppress the high levels of cortisol (Carroll et al., 1981) and also demonstrated to not inhibit adrenocorticotropic hormone to corticotrophin (Holsboer, 2000; Plotsky et al., 1998). Although a number of studies have found evidence for possible increased HPA axis activation in BD, the specific sites of abnormalities in the HPA axis in BD remain to be determined (Duffy et al., 2012; van der Werf-Eldering et al., 2012).

In the past decade, there has been a renewed interest in identifying peripheral biomarkers in patients with BD and a number of different approaches have provided compelling data. Of particular interest are studies that have examined three areas: cell growth and survival and synaptic plasticity, including brain-derived neurotrophic factor (BDNF); inflammation, especially the pro- and anti-inflammatory cytokines; and energy metabolism, particularly oxidative stress and mitochondrial function.

Brain-derived neurotrophic factor (BDNF)

Decreased peripheral levels (i.e. serum or plasma) of BDNF have probably been the most consistent finding in this area of research (Fernandes et al., 2011; Grande et al., 2010). The first report to find lower serum BDNF levels during mania and depression was published by Cunha et al. in 2006 and was quickly replicated (Cunha et al., 2006). Machado-Vieira et al. (2007b) reported decreased plasma BDNF levels in unmedicated patients with BD during a manic episode. De Oliveira et al. (2009) found decreased levels of BDNF in manic and depressive patients regardless of the medication status. In addition, BDNF has been described as an important mediator for cellular survival (Post, 2007), which raises the possibility that BDNF may also be involved with disease progression. To this point, Kauer-Sant'Anna et al. (2009) evaluated the peripheral levels of BDNF in patients with BD in the early (0–3 years) and late stage (10–20 years) of illness, and found decreased levels of BDNF only in patients in the late stage of illness. One study reported significantly decreased mRNA levels of lymphocyte-derived BDNF and decreased protein BDNF levels in platelets in manic unmedicated children and adolescents versus controls (Pandey et al., 2008). Two independent longitudinal studies conducted with manic patients found that peripheral BDNF levels increase after successful pharmacological treatment (de Sousa et al., 2011; Tramontina et al., 2009). In a recent systematic review of 13 studies including a total of 1113 subjects, Fernandes et al. (2011) found that BDNF levels were consistently reduced during manic and depressive episodes, but not during euthymia, and increased after treatment for acute mania. However, a preliminary 16-week open trial of quetiapine XR for BD found that peripheral BDNF levels increased in bipolar depression but decreased in manic/mixed patients, which suggested that the BDNF response differed depending on the polarity of illness (Grande et al., 2012). Not surprisingly, peripheral BDNF seems to be inversely associated with age and length of illness, supporting their potential importance in understanding illness progression (Yatham et al., 2009). Together these results support the potential use of peripheral BDNF as a biomarker for mood state and disease progression.

Inflammation

With respect to inflammation, a number of studies across different laboratories have also found that depressive and to a greater degree manic states are associated with increased peripheral levels of pro-inflammatory cytokines, such as interleukin (IL)-2, IL-6, IL-8 and tumor necrosis factor (TNF)-α (Brietzke et al., 2009; Kim et al., 2007; O'Brien et al., 2006). It has been demonstrated that manic patients with BD have increased IL-6 and TNF-α protein (O'Brien et al., 2006) and mRNA levels (Padmos et al., 2008) when compared to healthy controls. Interestingly, the profile of mRNA encoding for inflammatory genes in bipolar subjects overlaps with almost all genes associated with schizophrenia (Drexhage et al., 2010). Modern proteomics analysis also revealed that inflammatory pathways, including TNF-α, IL-13 and apolipoprotein A1 are associated with BD (Herberth et al., 2011). How lithium modifies these parameters has also been evaluated. For example, Sussulini et al. (2011) investigated the serum proteome signatures in lithium-treated BD patients and showed that patients taking lithium had increased apolipoprotein A1 expression in comparison to patients who were not taking the drug, suggesting lithium's potential involvement in modulating inflammatory responses.

Focusing on youth, recent preliminary findings from adolescents with BD also suggest that high-sensitivity C-reactive protein (hsCRP) is associated with manic symptom severity, and that serum BDNF protein levels are negatively associated with IL-6 protein levels (Goldstein et al., 2011). These findings support that inflammation might be associated with the illness progression and, therefore, support future investigations using large sample size and highlight the need for longitudinal studies incorporating repeated measures of these markers. While most of these studies excluded patients with inflammatory conditions such as asthma and allergies, for example, individuals with BD are at a higher risk to develop comorbid medical conditions including diabetes, metabolic disorder and

cardiovascular diseases (Weiner et al., 2011), which are also associated with elevated levels of the above-mentioned inflammatory markers (Leboyer et al., 2012). Longitudinal studies evaluating the levels of inflammatory markers and their relation with the development of medical comorbidities are essential in helping to understand the role of inflammation in BD.

Oxidative stress

Growing evidence has also shown that mitochondrial dysfunction and oxidative stress play an important role in the pathophysiology of BD (for review, see Berk et al., 2011). Oxidative stress is defined as an imbalance between antioxidants and oxidants, leaning towards increased levels of oxidants (i.e. free radicals). In a meta-analysis investigating biomarkers of oxidative stress in individuals with BD, Andreazza et al. (2008) found that thiobarbituric acidic reactive substances (TBARS), a marker of lipid peroxidation, and nitric oxide (NO) were significantly elevated in all phases of BD (Andreazza et al., 2008). Independent laboratories have shown increased levels of NO in patients with BD during mania (Gergerlioglu et al., 2007; Savas et al., 2002; Yanik et al., 2004), depression (Selek et al., 2008) and euthymia (Savas et al., 2006). Lipid peroxidation also was found to be increased in the different phases of BD (Andreazza et al., 2007), suggesting that lipid peroxidation and nitric oxide levels may be useful markers. In addition, preliminary data indicate that increased oxidative stress may be corrected with pharmacological treatment (Frey et al., 2007; Machado-Vieira et al., 2007a; Ozcan et al., 2004), although studies with larger sample sizes are needed to better test this issue.

A number of studies suggest that there may be a link between inflammation, oxidative stress and neuroplasticity pathways (Figure 1). For instance, inflammation has been shown as an important trigger of oxidative stress through activation of calcium-dependent proteins and direct inhibition of the mitochondrial electron transport chain (de Gonzalo-Calvo et al., 2010). Moreover, IL-6 has been associated with aging-dependent neurodegeneration and memory impairment (Godbout and Johnson, 2004). Feltes et al. (2011) demonstrated that IL-6 and TNF-α interact with proteins involved in neurodevelopment and neurodegeneration. Kapczinski et al. (2011) proposed a 'systemic toxicity index' composed by these dimensions: neurotrophins, oxidative stress markers and inflammatory markers. Using principal component analysis, they found that BD subjects during manic and depressive episodes had lower toxic indexes than patients with sepsis (used as positive controls) but higher than euthymic and healthy controls. In summary, peripheral biomarkers suggest: (1) that BDNF levels seem to vary with disease state and treatment response; (2) an imbalance between pro- and anti-inflammatory cytokines, towards an augmentation of pro-inflammatory cytokines, such as IL-6 and TNF-α; and (3) an increased oxidative stress, especially lipid peroxidation. Larger prospective studies with repeated measures are needed to determine whether peripheral biomarkers may predict course of illness, disease progression, or medication response.

Genetic biomarkers in BD

Genome-wide association studies in BD

Many strategies have been used to identify genetic biomarkers of BD, such as candidate-gene approaches or more recently genome-wide linkage and association studies. The former were based on the function of the gene and were most often restricted to a couple of common polymorphisms known to have a functional effect on the molecule properties. However, these studies were often limited to a small sample size and did not explore the influence of additional variants in the gene. This frequently resulted in false positive results with no replication on independent cohorts. Nevertheless, consistent results and significant meta-analyses reported association between polymorphisms located in the genes encoding BDNF, COMT and 5-HTT and BD; such associations were also found for other psychiatric disorders such as schizophrenia, unipolar depression, eating disorder, etc. No hypothesis-driven strategies based on the whole genome exploration were thus developed in order to identify relevant genetic biomarkers of BD. In particular, recent technological improvement allowed genome-wide association studies (GWAS) of large groups of patients and controls. These strategies, consisting of comparing allele frequencies between patients and controls for thousands of single nucleotide polymorphisms (SNPs) spanning the genome, require very large cohorts of subjects (thousands) in order to reach the genome-wide statistical significant threshold of 5×10^{-8}, as well as independent cohorts for replication and validation. Meta-analyses of these GWAS have been conducted and several candidate risk loci in BD have been identified, for instance in *CACNA1C* (alpha 1C subunit of the L-type voltage-gated calcium channel) and *ANK3* (ankyrin 3) (Ferreira et al., 2008; Liu et al., 2011; Schulze et al., 2009; Scott et al., 2009; Sklar et al., 2008; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). These loci have also been identified in non-caucasian populations (Takata et al., 2011). However, one limit in the increase of the number of subjects is to decrease the stringency of clinical criteria to constitute the group of patients and thus to limit the specificity of the results. Indeed, the *CACNA1C* locus has also been associated with schizophrenia (Athanasiu et al., 2010; Green et al., 2010; Nyegaard et al., 2010; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). Before their identification in GWAS, the functional roles of these genes were poorly understood. *CACNA1C* belongs to the family of L-type

calcium channels that mediate various calcium-dependent processes in neurons. It regulates the dendritic calcium influx in response to synaptic activity (Vacher et al., 2008). A missense *CACNA1C* mutation causes Timothy syndrome, with a phenotype including autism (Gargus, 2006). In mice, *CACNA1C* haploinsufficiency was associated with lower exploratory behavior, decreased response to amphetamine, and antidepressant-like behavior in the forced swim and tail suspension tests, with sex-specific effects (Dao et al., 2010). *ANK3* is a member of a family of ankyrin proteins. These proteins connect membrane proteins to the underlying spectrin-actin cytoskeleton, and play key roles in cell motility, activation, proliferation, contact, and the maintenance of specialized membrane domains. It is also known to modulate the activity of neuronal sodium channels (O'Donovan et al., 2009).

Studies that combine genetic and brain imaging allow the identification of neural systems that mediate heritable risk linked to these candidate common variants (Meyer-Lindenberg, 2010). For *CACNA1C*, several MRI studies have associated the risk variant to potential susceptibility mechanisms. CACNA1C rs1006737 risk allele (G to A) was reported to be associated with higher gray matter volume (Kempton et al., 2009) and increased gray matter density in the right amygdala and hypothalamus (Perrier et al., 2011), with some equivocal results (Franke et al., 2010). Regarding functional imaging, the same risk allele seems to be associated with increased limbic activity during an emotional or reward task in fMRI (Bigos et al., 2010; Jogia et al., 2011; Wessa et al., 2010). There is no MRI data for *ANK3* but the *ANK3* risk allele was associated with reduced sensitivity in target detection during sustained attention (Ruberto et al., 2011). In summary, the use of GWAS in very large cohorts allowed, without a prior hypothesis, the identification of at least two potential biomarkers of BD, *CACNA1C* and *ANK3*. Imaging studies are beginning to unravel the functional correlates and susceptibility mechanisms of these variants.

Polygenic exploration of BD

Another interesting approach to identify biomarkers has been developed by the International Consortium on Schizophrenia (ICS) and also relies on findings from GWAS (ICS, 2009). This approach not only considers SNPs showing the biggest difference in allele frequency between cases and controls, but also the aggregate effect of common variants in the context of a polygenic model. In this study, a large list of polymorphisms (approaching 74,062 polymorphisms), which had the lowest *p*-values, were selected. A score for each individual was calculated in an additive model, according to the genotype weighted by the odds ratio identified for the selected SNPs. When comparing the mean score for subjects, they showed a significantly higher score for patients with schizophrenia compared with unaffected controls ($p = 2 \times 10^{-28}$), suggesting a strong polygenic contribution in the vulnerability to schizophrenia, explaining at least one-third of the total variation in liability. Interestingly, the authors replicated these data using the same dataset of SNPs on two independent additional cohorts of patients with schizophrenia and controls, but also on two independent cohorts of patients with BD and unaffected controls, suggesting that these polygenic vulnerabilities might be common to schizophrenia and BD. By contrast, those results were not replicated in non-psychiatric diseases, such as coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis and type I and II diabetes, suggesting a relative psychiatric specificity for these biomarkers.

Transcriptomic approaches

Gene expression analysis has been widely used in BD to understand molecular mechanisms underlying the disorder. Many gene expression analyses have been performed comparing postmortem brains from patients and controls using more sophisticated microarray technologies (for review, see Sequeira and Turecki, 2006). These studies proposed genes in numerous pathways that may play a role in the pathophysiology of BD, including receptors, channels, transporters, stress response proteins, molecular chaperones, proteins involved in transcription or translation (Iwamoto et al., 2004), oligodendrocyte functions (Tkachev et al., 2003), neural network (Nakatani et al., 2006), ubiquitin cycle and synaptic functions (Ryan et al., 2006). Although working on the same postmortem brain samples from the Stanley Medical Research Institute, investigators screened different brain regions, suggesting the existence of region-specific changes in gene expression in the brain of subjects with BD. Studies focused on peripheral samples have been fewer in numbers. Tsuang and colleagues looked at blood samples from 30 patients with schizophrenia, 16 patients with BD and 28 unaffected controls. They identified 89 genes that allowed discrimination between the three groups of subjects, with an overall accuracy of 95–97%, suggesting that these biomarkers may have a significant diagnostic value (Tsuang et al., 2005). Several more recent studies allowed good discrimination of patients with BD by studying the expression of inflammatory genes (Padmos et al., 2008) or a set of three genes including *ANK3* (Kato et al., 2011). The transcriptomic approach also revealed that the transcriptional level and the amplitude of the rhythmic expression of several circadian genes were reduced in fibroblasts from bipolar patients (Yang et al., 2009). These findings deserve further investigation and replication. Although no consensus has get emerged from gene expression studies to date, the overall data reveal patterns of expression with potential both in terms of diagnostic utility as well as in revealing new biological insights into BD.

A convergent functional genomic approach

A novel approach to identify genetic biomarkers in BD has been proposed by Le-Niculescu and colleagues. They used complementary approaches in a convergent functional genomic strategy by the integration of data from genomics on human and animal models and of multiple independent lines of evidence converging on the same gene to decrease the signal-to-noise ratio and prioritize candidate genes (Le-Niculescu et al., 2009). By integrating data from linkage analysis, GWAS gene expression data from human postmortem, blood samples and animal studies, they found a list of candidate genes including *ARNTL*, *BDNF*, *ALDH1A1* and *KLF12*, which are respectively involved in regulation of circadian rhythms, neurotrophic function, brain development and transcriptional repression. The model that emerges from this work suggests that several molecular mechanisms are involved in the pathophysiology of BD, with some overlap in schizophrenia.

Little research has been conducted regarding genetic factors implicated in pediatric BD, despite the fact that there may be a particularly large genetic contribution in these early-onset cases (Faraone et al., 2003), and substantial evidence that pediatric BD is highly familial and heritable (Birmaher et al., 2009; Geller et al., 2006). Although linkage disequilibrium of the BDNF val66met, glutamate decarboxylase 1 (GAD1) and dopamine transporter (DAT) polymorphisms have been reported, replicated findings are still lacking (Geller et al., 2004; Mick and Faraone, 2009). No significant associations have been found between genetic polymorphisms and clinical features such as antidepressant-induced mania (HTTLPR) (Baumer et al., 2006; Biernacka et al., 2012) or ultradian cycling (COMT) (Geller and Cook, 2000); however, most of these studies are small and underpowered.

Conclusions

Biological research in the field of psychiatry in general and BD in particular has generated new insights into the mechanisms associated with risk, disease expression and treatment response. Brain imaging studies suggest that brain connectivity within prefrontal cortical areas and the limbic system may be of particular interest to studies of biomarkers in BD. Studies evaluating peripheral biomarkers point out three main areas as potential candidates: oxidative stress, inflammation and neurotrophins, especially BDNF (Figure 1, panel 2). Finally, genetic studies have associated alteration in genes related with calcium, circadian rhythm, cell growth/development and brain connectivity with BD. These new insights highlight the underlying complexity in the pathophysiology of BD but also signal the ability to apply translational approaches to diagnosis and treatment. Although some of these promising biomarkers may not survive rigorous empirical testing they may still inform our thinking about the biological underpinnings of BD. Biomarkers additionally have promise in guiding the development of novel therapies active on those targets (Berk et al., 2008; Magalhaes et al., 2011; Pasco et al., 2010).

Key unanswered questions relate to the usefulness of these biomarkers in predicting outcome at the earlier stages of the illness and guiding treatment options. For example, the findings outlined above strongly suggest that neuroprotection may need to be considered as a therapeutic target in addition to symptomatic control (Berger et al., 2003). This line of thinking also suggests a reformulation of the outcome measures and monitoring of treatment intervention. It is also possible that biomarkers related to disease staging can help us understand aspects of the pathophysiology of the disorder as it unfolds over time. If disease-related changes in biomarkers can be reversed with treatment then one might consider that at least some disease mechanisms are modifiable and amenable to interventions leading to secondary prevention. A correlate of this is the potential of using biomarkers to identify high-risk individuals who are likely to convert from the asymptomatic to the syndromal stage, thus aiding in very early identification of people likely to convert to syndromal disease expression. Future studies adding biomarkers to large-scale, prospective cohorts, as well as to well-designed RCTs, will be extremely valuable in ultimately determining the usefulness of these markers in a more rigorous scientific approach.

The lessons from other fields of medicine may show us ways forward that can bring laboratory tests and biomarkers right to the forefront of modern psychiatry. This would bring psychiatry closer to other fields of medicine, where bridging bench knowledge to bedside has been achieved by the search and development of useful biomarkers.

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