The neurobiology of bipolar disorder: identifying targets for specific agents and synergies for combination treatment

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Abstract
Bipolar disorder (BD) is a chronic psychiatric illness described by severe changes in mood. Extensive research has been carried out to understand the aetiology and pathophysiology of BD. Several hypotheses have been postulated, including alteration in genetic factors, protein expression, calcium signalling, neuropathological alteration, mitochondrial dysfunction and oxidative stress in BD. In the following paper, we will attempt to integrate these data in a manner which is to understand targets of treatment and how they may be, in particular, relevant to combination treatment. In summary, the data suggested that BD might be associated with neuronal and glial cellular impairment in specific brain areas, including the prefrontal cortex. From molecular and genetics: (1) alterations in dopaminergic system, through catechol-O-aminotransferase; (2) decreased expression and polymorphism on brain-derived neurotrophic factor; (3) alterations cyclic-AMP responsive element binding; (4) dysregulation of calcium signalling, including genome-wide finding for voltage-dependent calcium channel α-1 subunit are relevant findings in BD. Future studies are now necessary to understand how these molecular pathways interact and their connection to the complex clinical manifestations observed in BD.

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Introduction
Bipolar disorder (BD) is a chronic psychiatric illness reported to be responsible for the loss of more disability-adjusted life-years than all forms of cancer and major neurological conditions (Altamura et al., 2011; Merikangas et al., 2011). BD has been demonstrated to have four times higher health care costs than the general population. This has made BD a leading health concern (Altamura et al., 2011; Merikangas et al., 2011). Over the last five decades, there has been extensive research to understand the aetiology and pathophysiology of BD. Several hypotheses have been postulated, including alteration in calcium signalling (Kato, 2008), inflammatory response (Goldstein et al., 2009a), decreased density and size of neurons and glia (for review, see Gigante et al., 2010) and genetic factors (Schulze, 2010). In recent times there has been a growing focus on the involvement of mitochondrial dysfunction and oxidative stress in BD (Andreazza et al., 2008; Clay et al., 2010; Konradi et al., 2012). In the following paper, we will attempt to integrate these data in a manner which is to understand targets of treatment and how they may be, in particular, relevant to combination treatment. First, the neuropathology of BD is described. Next, several pathways that are supported both by findings from genetic findings and evidence of changes in protein levels or function will be described. This is followed by work that is of particular interest to our lab on mitochondrial dysfunction. The paper concludes with a concise discussion of how these data might be relevant to current treatment modalities and those that may hold promise in the future.
Cellular damage occurs in the brain in patients with BD

There are many questions that continue to remain unanswered regarding the neurochemical mechanisms and neuropathology that underlie BD; specifically, how such abnormalities in the brain translate into the symptoms of mood disorders. Structural neuroimaging and postmortem studies have highlighted anatomical and neuropathological abnormalities in patients with BD. These include ventricular enlargement, reduction in grey matter volume, decreased levels of markers for neuronal integrity, such as N-acetyl-aspartate, and reduction of neuronal and glial cell density (Rajkowska et al., 2001; Lopez-Larson et al., 2002).

The cumulative evidence from brain imaging studies, supporting structural and functional changes in BD, has raised intriguing questions. Postmortem studies have allowed us to examine the components of specific neuronal circuits at the cellular and molecular levels (Rajkowska, 2003). A significant decrease in the number or density of neurons in several brain areas, including the prefrontal cortex (Ongur et al., 1998; Rajkowska et al., 2001), anterior cingulate cortex (Benes et al., 2001b), hippocampus (CA2; Benes et al., 1998, 2001a), hypothalamic paraventricular nucleus (Manaye et al., 2005) and amygdala (Bezchlibnyk et al., 2007) have all been reported. Additionally, several studies have reported low glial cell number in the PFC (Ongur et al., 1998). For example, Rajkowska et al. (2001) has reported decreased glial cell size in the dorsolateral PFC of patients with BD. Bowley et al. (2002) did not observe alterations in the overall neuronal or glial in the amygdala between BD patients and controls but found a significant reduction of glial density and number in a small subset of BD patients who had not used lithium or valproate. This suggested that these mood stabilizers might affect glial cytoarchitecture, at least in this particular brain region (Bowley et al., 2002). Thus, postmortem studies have suggested that BD might be associated with neuronal and glial cellular impairment in specific brain areas, including the PFC, which are supported by findings from genetic and protein alterations, as described in the next section. Dysregulation of dopaminergic, neurotrophic and calcium pathways are known to impair mitochondrial functionality, which induce cellular damage through increasing oxidative damage to protein, lipids and DNA, culminating in apoptosis activation (Halliwell and Gutteridge, 2007; Kato, 2008; Clay et al., 2010; Konradi et al., 2012).

Several target genes identified in association studies are supported by changes in protein levels and cellular function

Genetic factors are believed to play a role in the aetiology of BD, yet the results are not consistent enough to build a genetic risk factor for the manifestation of the disorder (Schulze, 2010; Schulze et al., 2012). A full discussion of the genetics of BD is beyond the scope of this paper but covered by a number of excellent reviews (Schulze, 2010; Brennand et al., 2012; Schulze et al., 2012; Toker et al., 2012). Nonetheless, evidence accumulated thus far supports a number of interesting gene targets, which may be particularly relevant to the neurobiology of BD. Linkage and genome-wide association studies (GWAS) have identified genes associated with BD, generating feasible candidate genes. From linkage studies, catechol-O-aminotransferase (COMT), brain-derived neurotrophic factor (BDNF) and cyclic-AMP responsive element binding (CREB) proteins have been identified as candidate genes (for review, see Schulze, 2010; Byerley and Badner, 2011; Sears et al., 2011). GWAS in BD have revealed that ankyrin-G (ANK3; Baum et al., 2008; Ferreira et al., 2008; Scott et al., 2009; Smith et al., 2009; Liu et al., 2011) and voltage-dependent calcium channel α-1 subunit (CACNA1C; Ferreira et al., 2008; Sklar et al., 2008, 2011; Nishioka et al., 2012) are the strongest common associations in BD. ODZ4, a gene involved in cell signalling has also been identified as a potential candidate (Group, 2011; Green et al., 2012). More detailed findings for each of the genes mentioned above are described below. Figure 1 summarizes these findings. In the next section, we describe several of this gene targets especially as they are associated with findings on differences in protein expression and function, which may be important to understanding treatment.

Catechol-O-aminotransferase

The first pathway is supported by linkage studies to the COMT gene. One of the catecholamines of particular interest to BD is dopamine. The dopaminergic system has been extensively studied in BD (for review, see Berk et al., 2007; Cipriani et al., 2011). For instance, studies from the 70s and 80s decades have shown that antipsychotics can block the mania-like effect induced by amphetamine in healthy individuals (Jonsson et al., 1972; Anggard et al., 1973; Halbreich and Endicott, 1981; Nurnberger et al., 1982; Silverstone and Cookson, 1983). Additionally, Cipriani et al. (2011), in a recent meta-analysis, demonstrated that antipsychotics are highly effective and tolerate as
treatment for mania episodes, reinforcing the role of dopamine in BD. Dopamine is metabolized by two enzymes monoamine oxidase and COMT (Napolitano et al., 1995). This review will further explore the COMT role in BD due to its findings from linkage (Shifman et al., 2004; Lelli-Chiesa et al., 2011) and epigenetics (Abdolmaleky et al., 2006; Nohesara et al., 2011) studies. COMT is the enzyme responsible for converting extracellular dopamine into 3-methoxytyramine or 3,4-dihydroxyphenylacetic acid into homovanillic acid (Lachman et al., 1996; Fig. 1). The COMT gene is located on chromosome 22q11 (Dickinson and Elvevåg, 2009). Interestingly, a small deletion on region 11 causes a syndrome known as 22q11.2 deletion syndrome characterized by the presence of one copy of COMT per cell when the usual is two copies per cells (Dickinson and Elvevåg, 2009). Hence, this syndrome leads to degradation of the neurotransmitter in the PFC, which potentially puts these patients at risk of developing psychiatric illnesses (Dickinson and Elvevåg, 2009). The most widely studied COMT variant is a functional polymorphism of COMT Val<sup>158</sup>Met (rs4680) that has been shown to significantly decrease the activity of COMT in the PFC (Weinshilboum et al., 1999; Chen et al., 2004). Studies have also demonstrated an association of this polymorphism with BD (Shifman et al., 2004) and with reduction of cortical efficiency in the ventrolateral PFC in individuals with affective morbidity (Lelli-Chiesa et al., 2011). COMT has also been a target for methylation changes in BD. Abdolmaleky et al. (2006) found hypomethylation in the promoter region of COMT in the frontal lobe of patients with BD as compared to controls. More recently (Nohesara et al., 2011) demonstrated hypomethylation in the promoter region of COMT DNA in saliva from patients with BD. Moreover, COMT Val<sup>158</sup>Met and DRD3 Ser<sup>9</sup>Gly polymorphisms have been found to interact in samples of patients with BD type I but not in patients with BD type II (Lee et al., 2011). A recent review has refocused our attention on the effectiveness of dopamine-blocking drugs in mania (Cipriani et al., 2011), which makes this system of particular interest to understanding the neurobiology of BD.

![Diagram](http://ijnp.oxfordjournals.org/)
Cyclic adenosine monophosphate response element-binding

Another target supported by genetic linkage studies is a transcription factor – CREB (Sears et al., 2011). Cyclic adenosine monophosphate (cAMP) is part of a signal transduction pathway responsible for transcription regulation. Ligand activation of G-protein coupled receptors on the cell surface triggers this cAMP pathway, a cascade of biochemical signalling events that terminate when CREB proteins are phosphorylated (Mamdani et al., 2008). In order to regulate gene transcription CREB proteins, in their phosphorylated state, bind to the cAMP responsive element (CRE) recognition sequence of cAMP responsive genes (Mamdani et al., 2008). A number of studies have concluded that lithium decreases CREB phosphorylation, which can lead to decreased DNA binding and, in turn, to altered expression of cAMP responsive genes in the post mortem brain in patients with mood disorders (Bezchlibnyk and Young, 2002). Investigating CREB genes, from a sample of lithium responsive patients with BD, has led to the conclusion that two single nucleotide polymorphisms (SNPs), in genes belonging to the CREB family, are believed to be associated with BD and/or response to lithium (Mamdani et al., 2008). Therefore, changes in gene expression are likely to be involved in the mechanism of action of lithium treatment. In the near future this will help in the possibility of understanding lithium’s mechanism of action as a mood stabilizer and the genetic factors influencing its pharmacological efficacy.

Brain-derived neurotrophic factor (BDNF)

Another interesting pathway associated with BD and supported by linkage and protein expression studies is the BDNF (Kapczinski et al., 2010, 2011; Fernandes et al., 2011). The study of neurotrophins has received much interest in psychiatry, particularly because of their involvement as it induces survival, differentiation and development of cells (mainly neurons). BDNF has been the most studied neurotrophin in psychiatry illnesses (for review, see Kapczinski et al., 2010). Decreased serum levels of BDNF have been found in patients with BD during manic or depressive phases as suggested by meta-analysis from Fernandes et al. (2011). The BDNF gene is located on chromosome 11 and has nine functional promoters – 11 exons and three introns with several polymorphisms identified to date (Pruunsild et al., 2007; Sears et al., 2011). The most common polymorphism associated with BD is the Val66Met polymorphism (rs6265). It has been demonstrated to lead to alterations in hippocampal volume, associated with lithium response (Rybakowski et al., 2005, 2011; Rybakowski, 2011). More recently, Sears et al. (2011), in a family-based study, demonstrated an association of Val66Met with BD. This latter group, however, also identified a link between SNPs rs1519480, rs12227363 and rs11030107 of BDNF and BD (Sears et al., 2011). The SNPs rs1519480 and rs12227363 have been previously studied and demonstrated to have a relationship with BD (Liu et al., 2011). Moreover, BDNF Val66Met and COMT Val108Met polymorphisms were found to interact with patients with BD type II (Lee et al., 2013) adding complexity to the specificity of these genetics’ modification for BD.

CACNA1C

One of the genes with the strongest support from GWAS in BD is CACNA1C. Furthermore, a strong association with BD has recently been replicated in GWAS analysis combining several large data sets (Green et al., 2012). CACNA1C is a gene that encodes for the α1 subunit of an L-type voltage-dependent calcium channel. It is a large gene located on chromosome 12 region q13.3 including 44 invariant and six alternative exons with a coding region of >8 kb. Beitelshes et al. (2009) described a very low level of linkage in the region of the genome encoding CACNA1C, making SNP approaches for association studies of this gene problematic. Several studies have demonstrated an interesting connection of CACNA1C SNP Rs1006737 A/A with higher grey matter volume in patients with BD (Kempton et al., 2009), increased grey matter density in the right amygdala and hippocampus of BD with some equivocal results (Bigos et al., 2010) and increased limbic activity during an emotional or reward task in functional magnetic resonance imaging in patients with BD. Moreover, a recent study reports that the CACNA1C risk allele (rs1006737) has a negative relationship with cognition measures in patients with BD (Arts et al., 2013). Calcium (Ca2+) influx controlled via L-type channels is strictly regulated in order to maintain physiological intracellular calcium levels (De Jongh et al., 1996). To date, it is still not clear if the polymorphism (rs1006737) associated with BD affects the functionality of this channel leading to altered regulation of calcium influx. However, elevated intracellular Ca2+ has been recognized as a marker of BD (Altamura et al., 2011). More specifically, Dubovsky et al. (1994) were the first to report that intracellular calcium concentration in platelets was elevated in patients with BD. Subsequently, the same group found increased free intracellular calcium ion concentrations in the cells of patients afflicted with
both depression and mania (Dubovsky et al., 1994). Increased total serum and ionized calcium have also been reported in euthymic lithium-treated patients compared to healthy controls (El Khoury et al., 2002). In addition, elevated basal calcium concentrations have been detected in transformed B lymphoblasts in BD compared with those with BD-II, major depression or healthy controls (Emamghoreishi et al., 2000). Thus, calcium homoeostasis appears altered across all mood states in BD (for review, see Langan and McDonald, 2009). Ca²⁺ is a very interesting molecule that deserves further investigation in BD. The involvement of Ca²⁺ has already been demonstrated in genetics, brain organization and intracellular signalling of BD.

Summary

The above results highlight several pathways and systems that may be relevant to understanding the pathophysiology of BD, including the dopaminergic system, through polymorphism and epigenetic modulation of COMT, impaired cell survival due to decreased levels and genetic modification of BDNF and ANK3 and calcium metabolism, as demonstrated by increased intracellular calcium and a mutation ofCACNA1C. These potential molecular pathways may interact together and lead to the complex clinical manifestations observed in BD and the need for individualized and complex pharmacotherapy.

It is of particular interest that these findings are very much in line with the broader literature on the neurobiology and treatment of BD (Gould et al., 2004; Jope and Roh, 2006; Zarate and Manji, 2008; Kim et al., 2010; Machado-Vieira et al., 2011; Uemura et al., 2011). For example, studies have shown that lymphoblast cell lines from patients with BD carrying the aberrant allele AA presented significantly reduced Bcl-2 messenger RNA and increased levels of cytosolic calcium, which can be reverted by lithium treatment (Machado-Vieira et al., 2011). In support, Kim et al. (2010) have demonstrated decreased mRNA and protein levels of Bcl-2, as well as increased levels, mRNA and protein, of pro-apoptotic protein (Bax and BAD) in post mortem PFC from patients with BD. Additionally, glycogen synthase kinase 3 (GSK-3), a protein that when activated promotes intrinsic apoptosis, has been identified as an important target of lithium action (Gould, 2006; Rowe et al., 2007). Lithium induces phosphorylation of GSK-3β, which blocks this enzyme, therefore preventing apoptosis (Rowe et al., 2007). Notably, the neuroprotective effects of lithium against excitotoxicity are mimicked by treatments with other GSK-3 inhibitors or by transfection with GSK-3 isoform-specific siRNA and dominant-negative mutants (Liang et al., 2008), suggesting that at least this aspect of lithium’s action is mediated through GSK-3 inhibition. Interestingly, sensitization of the cell to apoptosis, decreasing neurotrophic factors and dysregulating calcium levels together induce to mitochondrial dysfunction, as summarized in Fig. 1 and review by Gigante et al. (2010). Our laboratory focused on understanding the causes, consequences and relevance of mitochondrial dysfunction to BD. Therefore, in the next section we will review and summarize the main findings in this area, which are intriguing and fascinating, at least in our view.

Mitochondrial dysfunction and oxidative stress in BD

Mitochondria (MTC) are essential organelles for the generation of adenosine triphosphate in cells. This occurs through the transfer of electrons along the mitochondrial electron transport chain (mETC), creating a proton gradient and generating energy in the form of ATP. This process is called oxidative phosphorylation (Halliwell and Gutteridge, 2007). Oxidative phosphorylation begins in complex I, or nicotinamide adenine dinucleotide (NADH) dehydrogenase complex, which catalyses the transfer of electrons from reduced NADH to coenzyme Q (also called ubiquinone; Halliwell and Gutteridge, 2007).

During the transfer of electrons through the different ETC complexes, under physiological conditions, single electrons can escape and result in a single-electron reduction of O₂ leading to the formation of a superoxide anion (O₂⁻; Farris et al., 2005). Superoxides and several others reactive oxygen species (ROS) are pivotal in physiological processes, such as signal transduction (for review, see Farris et al., 2005; Valko et al., 2007). Pathological situations where mETC is dysfunctional can lead to generating excessive ROS. If ROS production overcomes the antioxidant defence system, cells will enter in a process known as oxidative stress damage. In this stage DNA, lipids and proteins are vulnerable to suffer oxidative damage (Halliwell and Gutteridge, 2007).

Converging lines of evidence have consistently demonstrated down-regulation of many mRNAs coding for mETC subunits (Sun et al., 2006; Vawter et al., 2006) and antioxidant enzymes genes in this disorder using microarray techniques in post mortem brain (Benes et al., 2006). Specifically, Konradi et al. (2004) showed that expression of many mRNAs coding...
for complexes I–V subunits were decreased significantly in the post mortem hippocampus of patients with BD, but not in those with schizophrenia. In a previous study from our group (Sun et al., 2006), we reported down-regulation of eight mitochondrial ETC-related genes. Using real-time quantitative PCR we further confirmed that mRNA levels of complex I subunit NDUFS7 and NDUFS8 was decreased. In the same line, Iwamoto et al. (2005) demonstrated decreased NDUFS8 and NDUFS1 mRNA levels in patients with BD. Moreover, Cheng et al. (2006), using genome-wide linkage scans, suggested linkage of chromosome 19p13 in BD. Interestingly, the NDUFS7 is located on the same chromosome.

The influence of oxidative stress in BD can be supported from several lines of evidence. For example, Benes et al. (2006) found that superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione-S-transferase and other genes associated with anti-oxidant reactions were down-regulated in the hippocampus of patients with BD, but not in schizophrenia. Alterations in antioxidant enzyme system have been verified in BD, especially in peripheral systems such as serum, plasma or red blood cells (Kuloglu et al., 2002; Ranjekar et al., 2003; Savas et al., 2006; Andreazza et al., 2007, 2008; Gergerlioglu et al., 2007; Machado-Vieira et al., 2007; Selek et al., 2008).

SOD activity has been reported to be increased in BD patients (Abdalla et al., 1986; Kuloglu et al., 2002; Savas et al., 2006; Andreazza et al., 2007; Machado-Vieira et al., 2007). Further, Andreazza et al. (2007) found that this increase occurred during the manic and depressed phases of BD but not in euthymia. This is corroborated in part by Machado-Vieira et al. (2007), who reported increased activity of SOD in unmedicated, manic BD patients. However, Savas et al. (2006) identified increased SOD levels in 27 euthymic BD and Gergerlioglu et al. (2007) reported decreased levels of SOD in 29 manic patients. The catalase activity was decreased in euthymic patients (Andreazza et al., 2007) and increased in unmedicated, manic patients (Machado-Vieira et al., 2007). Kuloglu et al. (2002) also found decreased levels of catalase in BD patients. Moreover, glutathione peroxidise (GPx) has also been reported in BD. Andreazza et al. (2007) demonstrated that euthymic BD patients have increased activity of GPx. However, other studies did not find any differences compared to a control group (Abdalla et al., 1986; Kuloglu et al., 2002; Ranjekar et al., 2003).

Additionally, an increased level of 4-hydroxynonenal, a lipid peroxidation marker, was found in the cingulate cortex of patients with BD (Wang et al., 2009). The oxidative damage to proteins is expressed in peripheral cells and brain systems. Andreazza et al. (2009) have reported increased levels of 3-nitrotyrosine in patients with BD in early (0–3 yr of illness) and late (10–20 yr of illness) stages of the disorder. Recently, our group extended the previous knowledge of decreased mRNA levels of NDUFS7 subunit in PFC of patients with BD, by measure (in the same brain region) the NDUFS7 protein levels, the complex I activity and the consequent oxidative (carbonyl levels) and nitrosative (3-nitrotyrosine) damage to proteins. We showed decreased NDUFS7 protein levels and complex I activity, followed by increased protein oxidation and nitration in PFC of patients with BD (Andreazza et al., 2010).

Accumulation of oxidative damage to MTC proteins might induce cellular death as a result of aggregation of oxidized protein, which may result in neurodegeneration (Murray et al., 2003). A complex I deficiency may sensitize neurons to mitochondrial-dependent apoptosis in response to pro-apoptotic protein, Bax, through mitochondrial oxidative damage to cardiolipin, a mitochondrial specific phospholipid, which releases cytochrome c within the mitochondrial intermembrane space and consequently activates programmed cell death through caspase 3 and 9 (Yin and Zhu, 2012). In addition, Benes et al. (2006) showed that apoptotic genes (FAS, BAK and APAF-1) are up-regulated in the hippocampus of individuals with BD. Together, these studies suggest that oxidative stress damage to protein, lipids and DNA is present in both the periphery and brain from patients with BD. Nonetheless, further study is required to identify which proteins are targets to carbonylation and nitration in patients with BD, which may provide new targets for development of neuroprotective strategies and may help better elucidate the pathophysiology of BD.

Implications for the pathophysiology of BD and treatment

The complexity of BD forms a great challenge to understand its pathophysiology. From post mortem studies, we learned that BD has neuronal impairment, as shown by decreased number of neurons in PFC (Ongur et al., 1998; Rajkowska et al., 2001); anterior cortex (Benes et al., 1998, 2001b) and hippocampus (Benes et al., 1998). Additionally, a decreased number of glial cells in PFC has also been reported in BD (Rajkowska et al., 2001). The molecular mechanism behind such deterioration, however, has not yet been
fully uncovered. As discussed above, COMT, CREB, BDNF, calcium channels (such as CACNA1C), mitochondrial dysfunction and oxidative stress are the most common molecular targets in BD. In this section, we will revise the molecular targets of mood stabilizers (lithium and valproate) and their impact on pathophysiology.

Factually, the hypothesis of dopamine hyperactivity during mania has been one of the most robust hypotheses in BD. This theory is based on the behavioural effects of dopamine agonists and antagonists. For instance, amphetamine produces euphoria that is similar to mania (Halbreich and Endicott, 1981; Silverstone and Cookson, 1983). Moreover, Cipriani et al. (2011) demonstrated in a meta-analysis that antipsychotics are the most efficient and tolerable among all the treatments available, which reinforces the importance of the dopamine system in BD. Above, we discussed polymorphism and epigenetic modulation of COMT. A meta-analysis by Zhang et al. (2009) also confirmed that the presence of the Met allele also makes an individual more susceptible to illness. Recently, Rybakowski et al. (2012) demonstrated that patients with BD are excellent lithium responders and are less prone to have Met/Met genotype. This suggests that individuals with BD carrying the Met allele would be associated with a worse prognosis. Fatemi and Folsom (2007) demonstrated that rats treated with clozapine, haloperidol and valproate presented decreased expression of COMT 24KDa, which led to increased dopamine levels and enhanced dopamine-dependent functions, such as working memory performance. For instance, it has been shown that COMT variants are associated with poor performance on measures of verbal memory in patients with BD when compared to healthy controls (Burdick et al., 2007) or cognitive dysfunction in mania or mixed episodes in patients with BD (Soeiro-de-Souza et al., 2012).

Over the years, multiple pharmacological treatments for BD have shown considerable efficacy in delaying the recurrence of mania or depression. Lithium is among those pharmacotherapies that have been proven effective and there is evidence that the response to lithium is familial and related to a family history of BD (Perlis et al., 2009). The response to long-term lithium treatment clusters in families of responders was significant. These findings also reported that the majority of relatives, of lithium responders, also benefitted from long-term administration of lithium (Grof et al., 2002). This suggests that there might be a genetic component or trait that can explain the efficacy of lithium as a mood stabilizer, yet GWAS of lithium response in BD have not yielded SNPs in the DNA that would meet the threshold for genome-wide association (Perlis et al., 2009).

Given the complexity of intracellular communication, several intracellular pathways have been implicated in the action of mood stabilizers (Coyle and Duman, 2003). For example, chronic lithium treatment changes CREB activity in excitotoxic situations by preventing loss of phosphorylated CREB and CRE-induced gene expression. Using a very well-characterized population of excellent lithium responders, Mamdani et al. (2008) carried out an association study of this sample with CREB1, CREB2 and CREB3 genes and found that CREB1-7H SNP (TT) is associated with lack of response to lithium. Dysregulation on the CREB system is of high relevance to BD, since cAMP signal transduction needs CREB to regulate gene expression (Bezhilinvyk and Young, 2002). More recent studies have suggested that mood stabilizers, especially lithium, can directly inhibit GSK-3β or GSK-3α by competing with Mg²⁺ or can indirectly inhibit it by modifying AKT signalling, which may account for the anti-apoptotic effects of the ion (Rowe et al., 2007; Freland and Beaulieu, 2012).

Studies have shown that lithium also targets BDNF (Chiu and Chuang, 2011). Suwalska et al. (2010) studied 141 euthymic patients with BD in long-term treatment with lithium in comparison to healthy controls (n=75). Patients were separated to excellent responder to lithium (n=30), partial responder (n=61) and non-responder (n=50). The results showed that, in general, patients have a lower level of lithium in comparison to controls. However, when stratified in groups, Suwalska et al. (2010) noted that only the non-responder group had low levels of BDNF, indicating that lithium might target BDNF as part of its mechanism of action. de Sousa et al. (2011) investigated the levels of BDNF before and after lithium monotherapy in patients with BD-type I. Lithium treatment (4 wk) was able to significantly increase the BDNF levels in patients. Although this was the preliminary stage of the study and only included 10 subjects, the results also suggested BDNF as a strong modulator of lithium’s action. Further studies are necessary to replicate this finding using a larger sample and with several occasions of lithium treatment to confirm whether BDNF is a target of lithium in BD.

Valproate has been considered a histone deacetylase inhibitor, which, consequently, induces DNA-demethylation, thus modulating gene expression. In this review, we will not cover the epigenetic mechanism of valproate (for review, see Guidotti et al., 2011). The combination of valproate and antipsychotics
is often used in clinics. Interestingly, Guidotti et al. (2011) showed that mice treated with valproate and clozapine exhibit a synergic action to induce demethylation. Also, Guidotti et al. (2011) did not observe improvement of chromatin remodelling for haloperidol or risperidone.

Additionally, mood stabilizers, especially lithium, have been shown to exert antioxidant capacity (Wang, 2007). Oxidative stress can be induced in neuronal cultured cells by mitochondrial complex I inhibition with rotenone or by direct application of hydrogen peroxide (H$_2$O$_2$; Wang, 2007). Cells under chronic lithium administration, and clinically relevant concentrations, are able to attenuate cell death induction by both rotenone and H$_2$O$_2$ (King and Jope, 2005; Lai et al., 2006). In the last few years, the neuroprotective effects of mood stabilizers against oxidative damage have begun to receive recognition, despite the fact that there is not enough data directly linking those effects to mood-stabilizer treatment for BD. If the pharmacological treatment for BD can be linked to the neuroprotective effects of drugs such as lithium, patients under long-term lithium treatment should manifest some of the antioxidant effects attributed to lithium and this should be able to prevent the oxidative damage linked to BD.

Findings have supported the increased Ca$^{2+}$ response and mutation in calcium channels (CACNA1C) in both peripheral blood cells and post mortem brain tissue of subjects with BD. This has been confirmed by several independent laboratories. Indeed, combination treatment such as lithium and verapamil, an L-type calcium channel blocker, has been shown to improve anti-manic efficacy in BD, as reported by Mallinger et al. (2008). Krupitsky et al. (2001) demonstrated that pre-treatment with nimodipine, an antagonist of L-type voltage-sensitive calcium channels, attenuated ketamine-induced euphoria and sedation, suggesting that medication that acts in the calcium channel might serve as a potential combination treatment for BD. Increased oxidative stress markers and decreased mitochondrial functionality have also been repetitively reported in BD (for review, see Clay et al., 2010). Intriguingly, an increase in intracellular levels of calcium is one of the main triggers to start an oxidative stress cascade (for review, see Berk et al., 2011). It is common knowledge that oxidative damage to proteins, lipids and DNA leads to cell loss and, therefore, it has been hypothesized that calcium$\times$oxidative stress interaction might be one of the possible mechanisms to explain cellular damage in BD. Future studies that include large samples are crucial to verify whether or not this relationship is true.

Another relevant target for BD is the glutamatergic system (Ginsberg et al., 2012; Machado-Vieira et al., 2012). This system exerts a central role in excitatory neurotransmission and neurodevelopment (Ginsberg et al., 2012). To review the mechanism of action of the glutamatergic system, see Machado-Vieira et al. (2012). Increased glutamate levels have been reported in several brain regions from patients with BD (Eastwood and Harrison, 2010; Machado-Vieira et al., 2012). Additionally, the NR1 and NR2A, subunits of ionotropic glutamate receptor, have been reported in BD (McCullumsmith et al., 2007). More recently, levels of vesicular glutamate transporter 1 were found to be increased in the anterior cingulate cortex from subjects with BD. These findings suggest that the glutamatergic system could be a potential target for the development of new treatment for BD as recently reviewed by Machado-Vieira et al. (2012). For example, ketamine, a non-competitive antagonist of the NMDA receptor, was demonstrated to ameliorate the depressive symptoms in patients with BD after 40 min infusion, the effects persist for up to 7 d (Diazgranados et al., 2010).

In addition to the pathways described above in this review paper, inflammation has been consistently reported in patients with BD, as recently reviewed by Goldstein et al. (2009b) and Leboyer et al. (2012). Augmentation of neuro-inflammation might be a response to increased levels of oxidative stress reported in these patients (Kupfer et al., 2011; Leboyer et al., 2012). It is worth mentioning that modulating neurotrophins, inflammatory system, oxidative stress, calcium, dopamine and glutamatergic system are all strategies to increase neuroprotection in patients with BD, as discussed by Berk et al. (2011), suggesting the potential for the development of new pharmacological strategies that act to prevent neuronal damage in patients at risk of developing BD.

Taken together, it is clear that the mechanism of action of mood stabilizers probably occurs in multi-target levels. Future studies are important to identify the connection between these targets and define how we can improve the action of mood stabilizers by combining new drugs, or those already existing, that act in the same signalling cascade and, hence, creating a synergic effect that might augment the drug effect.

Conclusion

We have reviewed a considerable body of evidence supporting abnormalities in genetic and protein regulation of cellular pathways, including neurotransmitters,
neurogenesis and oxidative metabolism that may result in cell impairment. In the past decade, neuroimaging studies have shown widespread cortical and subcortical involvement in BD, with grey and white matter loss. Evidence has also shown widespread biochemical pathway alteration in several regions in post mortem studies. Future studies are necessary to clarify which pathways are involved in which region. There is also considerable evidence that supports the involvement of oxidative damage related to mitochondrial dysfunction in BD. Future studies using oxyproteomic analysis could help us to understand which proteins are targets of oxidative modification. Therefore, oxidative damage could be used as a significant therapeutic target as well as a potential biomarker.

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Statement of Interest

None.

References


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