Oxidative Stress in Bipolar Disorder

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Abbreviations

5-HT	Serotonin
5meOHC	5-hydroxymethylcisteine
8-OHdG	8-hydroxy-2-deoxyguanosine
BD	Bipolar disorder
BER	Base excision repair
DNA	Glycosylase 1
ETC	Electron transport chain
GWAS	Genome-wide association studies
LPA	Lysophosphatic acid
MD	Mitochondrial dysfunction
MDD	Major depressive disorder
NER	Nucleotide excision repair
Ogg1	Oxyguanosine
PAF	Platelet-activating factor
PKA	Protein kinase A
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCZ	Schizophrenia

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1 Introduction

Bipolar disorder (BD) is characterized by mood fluctuations between episodes of mania and depression. It is also characterized by a larger loss of disability-adjusted life-years than all forms of cancer or major neurologic conditions (Merikangas et al. 2011), which elevates the health-care costs four times higher than that of the general population (Altamura et al. 2011). Therefore, BD is becoming a foremost health concern. The complex pathophysiology of BD has brought interest in several areas of research to investigate the causes and consequences of this mood fluctuation to the brain.

Several hypotheses have been postulated during this journey to identify the etiology and pathophysiology of BD, which includes inflammatory responses (Goldstein et al. 2009), genetic modifications (Schulze 2010), alteration in calcium signaling (Kato 2008a), and decrease of density and size of neurons and glia (for review see Gigante et al. 2010). Mitochondrial dysfunction, mitochondrial DNA abnormalities, protein expression of mitochondrial electron transport chain, reduced pH, and decreased levels of high-energy phosphates in the brain, as well as increased oxidative stress status, have been a common feature identified in several recent investigations carried out on patients with BD (Andreazza et al. 2008; Clay et al. 2010). Therefore, mitochondrial dysfunction and the consequent oxidative damage to biomolecules could be associated with the verified neuronal or glial impairment in BD (Beal 2002; Clay et al. 2010; Gigante et al. 2010). In the following section of this chapter, the most relevant results to date for BD and how calcium plays a role in this scenario will be discussed.

2 Evidence of Oxidative Stress in Bipolar Disorders

The central nervous system presents high amounts of oxidizable substrates, high oxygen tension, and relatively low antioxidant capacity making it extremely vulnerable to oxidative damage (Halliwell and Whiteman 2004; Sies 1991). When the cytoplasmic enzymatic and nonenzymatic antioxidant and mitochondria systems are overwhelmed by high levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), the DNA, lipids, and proteins can be damaged (Lenaz 2001).

Mitochondria are intracellular organelles that play a crucial role in ATP production carried out by the electron transport chain (ETC) complexes I, II, III, IV, and V in the inner membrane through a process known as oxidative phosphorylation (Chinopoulos and Adam-Vizi 2010). Mitochondria are not only essential for energy control but also for maintaining calcium homeostasis (Kato 2008b), regulating apoptosis, and generating reactive oxygen species (ROS) (Jeong and Seol 2008).

ATP production occurs through the flow of electrons along ETC complexes transferring protons across the inner membrane, producing a large mitochondrial membrane potential. The energy lost by the reentering protons in the mitochondrial matrix, through the ATP synthase protein, is used to form ATP (Green and Kroemer 2004; Lenaz 2001; Reeve et al. 2008). Single electrons escape during the ETC transfer, resulting in a single electron reduction of molecular oxygen forming superoxide anion (O_2^-), especially in complex I (NADH:ubiquinone oxidoreductase) (Green and Kroemer 2004). Superoxide dismutase (SOD) converts the mitochondrial O_2^- into hydrogen peroxide (H_2O_2), which in the presence of ferrous iron (Fe^{+2}) results in the production of hydroxyl radicals ($OH^\bullet$) via Fenton reaction ($H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + OH^- + OH^\bullet$). Another relevant event is the reaction of $O_2^{\bullet -}$ with nitric oxide ($NO^\bullet$), reactive nitrogen species produced by microglia and astrocytes, to form peroxynitrite ($ONOO^-$) (Naoi et al. 2005).

2.1 Protein Oxidation

Proteins can have their structure and functionality modified by oxidative damage (Beal 2002; Lee et al. 2009). In BD, many proteins are targets for oxidative damage, which may include synaptic function key proteins, such as synaptophysin (Mallozzi et al. 2009). The mitochondrial ETC proteins are more vulnerable to nitrosative damage, suggesting a functional relationship between mitochondrial dysfunction and nitrosative damage (Murray et al. 2003).

In the hippocampus of patients with BD, the neuronal nitric oxide synthase I, the enzyme that generates $NO^\bullet$, was found to be upregulated, in addition to increased serum levels of $NO^\bullet$ in subjects with the same disorder (Selek et al. 2008). Protein oxidative damage can be induced by reaction with hydroxyl free radical ($OH^\bullet$), which is catalyzed by Fe^{+2} and Cu^{+2}, introducing carbonyl groups (Beal 2002). Protein nitration occurs by reaction of $ONOO^-$ with sulfhydryl and hydroxyl residues (Naoi et al. 2005). Such modifications might inactivate the membrane signaling pathways and key enzymes (Naoi et al. 2005). The tyrosine residues nitration produces 3-nitrotyrosine in proteins that serves as a marker of $ONOO^-$ oxidative damage induced in vivo (Naoi et al. 2005). This oxidative damage affects protein functionality, altering, for example, enzyme activities (Beal 2002), and susceptibility to proteolytic degradation (Naoi et al. 2005).

Results from our group reported increased levels of 3-nitrotyrosine in postmortem prefrontal cortex (Andreazza et al. 2010) and also found increased serum levels of 3-nitrotyrosine in patients with BD in both early (0–3 years) and late (10–20 years) stages of the illness (Andreazza et al. 2009). In addition, other evidences give support to the vulnerability of mitochondrial protein to nitrosative damage. A functional relationship between complex I activity and nitration was shown in mitochondrial membranes from bovine heart, where $ONOO^-$ targeted mainly

complex I subunits, resulting in significant inhibition of complex I activity (Murray et al. 2003). This is further supported by the report by Naoi et al. (2005) of increased 3-nitrotyrosine levels in the mitochondrial complex I subunits, but not other mitochondrial proteins, and of SH-SY5Y cells incubated with $ONOO^-$. Other studies demonstrated increase of the oxidative stress markers such as protein carbonylation, lipid peroxidation, and 3-nitrotyrosine levels in the brain and peripheral blood cells of BD subjects (Machado-Vieira et al. 2007; Wang et al. 2009; Andreazza et al. 2009, 2010).

2.2 DNA Oxidation

DNA is also vulnerable to oxidative damage; hydroxyl radicals react with DNA causing single- or double-strand breaks (Halliwell and Gutteridge 2007) or promote oxidation to C-8 position of deoxyguanosine on DNA, forming 8-hydroxy-2-deoxyguanosine (8-OHdG). DNA oxidation can also be induced by $ONOO^-$, which forms strand breaks and base oxidation products and cause deamination of G and A leading to formation 8-nitro-deoxyguanosine (Burcham and Harkin 1999). DNA lesions are rapidly detected by the DNA damage response system (Barzilai and Yamamoto 2004). This response culminates in activation of cell-cycle checkpoints and the appropriate DNA repair pathways (Iliakis et al. 2003). Oxidative DNA damage is mostly repaired by base excision repair (BER) and nucleotide excision repair (NER) enzymes (Halliwell and Gutteridge 2007). Most of the damage is removed before the cell reach replication preventing damage transmission to new cells (Evans et al. 2000). If this system is overwhelmed by free radicals, mutations to adenine or cytosine (A:T to G:C or G:C to T:A transversion mutations) will occur and consequently activate the apoptosis machinery (Halliwell and Gutteridge 2007).

Recently, oxidative stress and deficiency of oxyguanosine DNA glycosylase 1 (Ogg1), an enzyme responsible to repair the damage resulting from 8-OHdG, are considered to be a crucial factor in the process of aging and aging-related diseases, such as Alzheimer (Mao et al. 2007). Mitochondrial oxidative phosphorylation subunits are assembled from proteins encoded in both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Complex I is assembled from 45 subunits, 7 of those are encoded by mtDNA and the rest by nDNA; complex II is formed from 4 nDNA polypeptides; complex III has 11 subunits and cytochrome C is encoded by mtDNA; and complex IV also has only one subunit from the 13 encoded by mtDNA (Wallace 2010). Therefore, to maintain an efficient energetic metabolism, the cells have to protect both mtDNA and nDNA from damage. Damage to mtDNA and nDNA can lead to various levels of mitochondrial dysfunction (loss of control, but still functional) and disorder (loss of functionality) (Campbell and Mahad 2012).

mtDNA mutation rate is usually higher than nDNA, which can be due to the lack of protection from histones and the proximity to mitochondrial ROS production (Wallace 2010). It is reasonable to expect that mtDNA repair system would be higher

than nDNA. On the other hand, the cells do not invest just enough energy to save the mtDNA till cell reproduction or death (Wallace 2007). Thus, it has been observed that mtDNA has higher levels of oxidation than nDNA (Bohr and Dianov 1999). The importance of mtDNA to keep a healthy CNS is highlighted by the number of mtDNA disorders, where complex II (nDNA) is spared. The section "Mitochondrial Dysfunction" of this chapter will describe the findings of mitochondrial DNA mutation in BD. Andreazza et al. (2008) have demonstrated an increased DNA fragmentation in lymphocytes from patients with BD during different episodes of the diseases. Additionally, Andreazza et al. (2008) reported a positive correlation between Young Mania Rating Scale (YMRS) and the intensity of DNA damage, highlighting the importance of illness severity for these findings. The technique used in this study was COMET assay, which is an easy method to detect DNA double- and single-strand breaks or damage. Interestingly, BD is associated with other known DNA damage diseases as cardiovascular, diabetes, and obesity. Che et al. (2010) found elevated oxidative damage to nucleic acids (8-hydroxy-2'-deoxyguanosine) in CA1, CA3, and dentate gyrus regions of the hippocampus among patients with BD, schizophrenia (SCZ), and major depressive disorder (MDD). Supporting the involvement of DNA damage in BD, Buttner et al. (2007) demonstrated increased DNA fragmentation in non-GABAergic neurons in postmortem anterior cingulate cortex from patients with BD. Finally the authors suggested that the increased DNA damage may be attributed to high oxidative stress associated with BD.

Telomere shortening has been a well-thought-out sign of growing oxidative stress and a marker of antioxidant defense capacity (Saretzki and Von Zglinicki 2002). Passos et al. (2007) utilized the replicative senescence model as a reliable cellular model of aging. He verified that mitochondrial generation of ROS is crucial for determining telomere shortening. To further support the accumulative effect of oxidative damage occurring in BD, Simon et al. (2006) found that the amount of telomere shortening in patients with chronic mood disorders corresponds to 10 years of accelerated aging. As Higuchi (2004) stated that oxidative DNA damage is an intermediate step to cellular apoptosis and knowing that telomeres are located in the end of mammalian chromosomes, it is believed that the link between mitochondrial ETC dysfunction, oxidative stress, DNA damage, and cell death is a promising field in the investigation of the pathophysiology of BD.

3 Sources of Oxidative Stress Damage in Bipolar Disorder

3.1 Mitochondrial Dysfunction

Mitochondrial dysfunction (MD) is a dysregulation of the ETC complex, which can be caused by genetic alteration; different toxins capable of inhibiting mitochondrial ETC complex, such as 6-hydroxydopamine, a sub-product of dopamine oxidation; or simply impaired activity (Halliwell and Whiteman 2004). BD may be associated with the susceptibility of oxidative stress; a downregulation of several complex I

subunits occurs in BD. Human complex I is composed of 45–46 different subunits and divided into three functional modules (dehydrogenase, hydrogenase, and transporter). Finally, the transporter module is responsible for translocation of protons across the membrane (Brandt et al. 2003). Interestingly, Iwamoto et al. (2004) and Sun et al. (2006) reported that NDUFV2, NDUFS1, NDUFS7, and NDUFS8 present decreased expression in BD in the dehydrogenase module. These results suggest that patients with BD may have reduced ability to oxidize NADH and to transfer electrons to ubiquinone. This means that O_2^- is produced because electrons may persist for sufficient time to react with molecular oxygen (Boveris and Chance 1973; Turrens and Boveris 1980; Andreazza et al. 2010). Together, the organization of complex I, downregulation of complex I subunits, and diminished antioxidant levels support the susceptibility of proteins from mitochondrial oxidative damage in BD. These oxidations on protein residues can alter protein function or lead to deleterious intermolecular aggregates (Beal 2002). Decreased expression of genes involved in proteasome degradation process was found in the prefrontal cortex of subjects with BD, suggesting a faster accumulation of carbonylated proteins (Konradi et al. 2004). The etiology and/or progression of several chronic central nervous system disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis, is associated with the accumulation of carbonylated proteins (Castegna et al. 2002a, b).

Many lines of studies suggest that MD plays a role in BD pathophysiology. In 1995, McMahon et al. (1995) speculated that mtDNA and imprinted DNA can be inherited from the mother and increase 1.3- to 2.5-fold the risk of illness for the offspring of affected mothers. Additionally, a deletion on mtDNA of 4,977 bp, known as the "common deletion," was reported to be associated with BD and SCZ. In the cortex of BD probands and suicide victims, significant increases in the prevalence of the common deletion were found (Kato et al. 1997). Kakiuchi et al. (2005) reported controversial results failing to find the association of common deletion levels with BD and schizophrenia individuals. Another genetic alteration is the polymorphism in the mt-ND1 gene (366T>C), supporting the involvement of mtDNA mutations in ETC functionality, associated with BD. The mt-ND1 alteration causes a decreased mitochondrial membrane potential and complex I activity (Munakata et al. 2004). A decreased mitochondrial matrix pH has been linked to 10398A>G polymorphism in BD and, also, to higher baseline and poststimulation mitochondrial Ca^{2+} levels (Kato and Kato 2000; Kato et al. 2003; Kazuno et al. 2006, 2008). Interestingly, mtDNA copy number can be modulated by the energy requirement for the cell. For example, Liu et al. (2003) reported that human leukocytes increase the mtDNA copy number in response to oxidative stress. Wallace (2007) reported that mutation in mtDNA accumulated with the aging process and might be a response to increment of oxidative damage during this process.

For instance, in BD, many mRNAs coding for electron ETC complexes I–V subunits, especially complex I, presented decreased expression (Clay et al. 2010). Postmortem hippocampus (Konradi et al. 2004) and frontal cortex (Iwamoto et al. 2005; Sun et al. 2006) revealed decreased expression of several mRNAs coding for ETC complexes I–V subunits by DNA microarray analyses. Iwamoto et al. (2005)

reported that mRNA levels in the prefrontal cortex of subjects with BD such as complex I subunit NDUFS1, complex III subunit UQCRC2, and complex IV subunit COX15 were decreased. Konradi et al. (2004) also identified nuclear mRNA coding for mitochondrial proteins and genes regulating oxidative phosphorylation and the adenosine triphosphate-dependent process of proteasome degradation. Sun et al. (2006) reported a downregulation of 8 mitochondrial ETC-related genes, using high-density cDNA spot microarrays, consisting of NDUFS7 and NDUFS8 (complex I), UQCRC2 (complex III), COX5A and COX6C (complex IV), and ATP5C1 and ATP5J (complex V) and confirmed that mRNA levels of NDUFS7 were decreased using real-time quantitative PCR. Moreover, evidence from several genotyping studies suggest that polymorphisms of complex I subunit NDUFV2 may be associated with BD, thus supporting the involvement of mitochondrial complex I dysfunction in BD (Xu et al. 2008; Washizuka et al. 2009). Moreover, other recent studies in subjects with BD have demonstrated alterations in a diverse set of oxidative stress parameters, such as alterations in antioxidant enzymes (Kuloglu et al. 2002; Savas et al. 2006; Andreazza et al. 2008), increased lipid peroxidation (Kuloglu et al. 2002; Savas et al. 2006; Machado-Vieira et al. 2007; Andreazza et al. 2008), increased DNA fragmentation (Andreazza et al. 2008; Buttner et al. 2007), and increased levels of nitric oxide (Savas et al. 2002; Selek et al. 2008) on peripheral blood cells. Oxidative damage modifies the structure and function of proteins, suggesting that such alterations might be connected with disease outcome (Beal 2002; Lee et al. 2009).

3.2 Calcium Metabolism

Several critical cellular responses are controlled by Ca^{2+}, which is a key element in signal transduction (Murray et al. 2003). Thus, Ca^{2+} ion levels are transported across the plasma membrane and the membranes of intracellular organelles through a number of tightly controlled channels, pumps, and exchangers. Calcium is mainly buffered by two organelles: endoplasmic reticulum and mitochondria (Adam-Vizi and Starkov 2010). Growing attention has been put toward increasing the understanding of the mechanisms involved in Ca^{2+} ion uptake by the mitochondria. Mitochondrial machinery is activated by the accumulation of Ca^{2+} leading to increased ATP synthesis and ATP levels in the cytosol (Rizzuto et al. 1999). The accumulation of calcium accelerates H^+ extrusion and activates oxidative phosphorylation (Hansford 1985; Santo-Domingo and Demaurex 2010), which can in turn increase ROS production (Adam-Vizi and Starkov 2010).

The ROS production induced by calcium can be triggered by different pathways including *(1)* activation of mitochondrial electron transport chain and thus increase the probability of leaking O_2^- in mitochondrial complex I and complex III; *(2)* stimulation of nitric oxide synthase (NOS) that increases the production of $NO^{\cdot}$ by catalyzing the oxidation of a guanidine nitrogen of L-arginine (L-Arg) producing L-arginine as an intermediate and $NO^{\cdot}$ as a final product; and *(3)* stimulation of

calcium-dependent endonucleases, which can induce DNA fragmentation, an important event in apoptosis. On the other hand, H_2O_2 can increase intracellular Ca^{2+} levels through the opening of TRPM2 cation channels resulting in cellular loss if not interrupted. The prolonged increase of Ca^{2+} can also induce mitochondrial permeability transition pore, leading to mitochondrial swelling and cytochrome C release resulting in cell death by apoptosis (Giorgi et al. 2012; Miller and Zhang 2011).

In BD, elevated intracellular Ca^{2+} and abnormal Ca^{2+} signaling have been recognized as markers (Kato 2008). The first report of elevated calcium concentration in BD was found in platelets (Dubovsky et al. 1994). Also, cells of patients afflicted with both depression and mania had increased free intracellular calcium ion concentrations (Dubovsky et al. 1994). Euthymic lithium-treated patients presented increased total serum and ionized calcium when compared to healthy controls (El Khoury et al. 2002). Besides, elevated basal calcium concentrations have been detected in transformed B lymphoblasts in BD-I compared with those with BD-II, major depression, or healthy controls (Emamghoreishi et al. 2000). In all mood states of BD, calcium homeostasis appears altered. Further, thrombin, serotonin (5-HT), and platelet activating factor (PAF) are agonist-induced calcium influx and are enhanced in cells derived from patients with BD regardless of the agonist used (Kato 2008). In peripheral blood cells of patients with BD, thapsigargin-induced cytosolic Ca^{2+} response was found to be increased (Hough et al. 1999; Kato et al. 2003; Perova et al. 2008). Perova and colleagues (2010) stimulated B lymphoblast cell lines from patients with BD-I with lysophosphatic acid (LPA), showing increased Ca^{2+} mobilization.

Another piece of evidence that supports the calcium involvement in BD come from genome-wide association studies (GWAS) and linkage studies. GWAS of large groups of patients and controls are a very promising strategy to identify relevant genetic biomarkers. GWAS meta-analyses showed that CACNA1C and ANK3 (ankyrin 3) are the major candidate risk loci in BD (Sklar et al. 2008; Ferreira et al. 2008; Kempton et al. 2009; Schulze et al. 2009; Bigos et al. 2010). CACNA1C gene encodes for the alpha-1 subunit of an L-type voltage-dependent calcium channel named Cav1.2. This gene, nearly 300 kb, including 44 invariants and 6 alternative exons with a coding region of over 8 kb is located on chromosome 12q13.3. Some studies showed in patients with BD intriguing associations between CACNA1C SNP Rs1006737 (A/A genotype) and higher gray matter volume (Kempton et al. 2009), increased gray matter density in the right amygdala and hippocampus with some equivocal results (Bigos et al. 2010), and increased limbic activity during an emotional or reward task in fMRI (Jogia et al. 2011).

Intracellular calcium levels are tightly regulated via L-type (cav1.2 and Cav1.3) calcium channels and consist of 24 transmembrane segments, which are activated by membrane depolarization and mediate cellular Ca^{2+} influx (Catterall 2000). Cav1.2 is a complex protein containing four subunits in the cardiac form (an α1 subunit of 190–250 kDa, a transmembrane disulfide-linked complex of α2 and δ subunits, a β intracellular subunit, and a γ transmembrane subunit) and three subunits in the neuronal form (α1, α2δ, β). The expression of voltage-dependent Ca^{2+} channels is regulated

through the phosphorylation pathway by a second messenger-activated protein (Catterall 2000). Ser1928 in the C-terminal domain is a target for phosphorylation through protein kinase A (PKA) (De Jongh et al. 1996) and plays a pivotal role in the functionality of Cav1.2, at least, in the cardiac isoform, as α1C subunit was found to bind to calmodulin, modulating Ca^{2+}-dependent inactivation and facilitation of the channel (Kameda et al. 2006). Supporting the integration between mitochondria and calcium channels, Koh et al. (2003), using myocytes from cerebral arteries, demonstrated that mitochondria sense IP3R-mediated sarcoplasmic reticulum Ca^{2+} release to control NF-κB-dependent Cav1.2 channel expression.

4 Concluding Remarks: Perspectives of Oxidative Stress

As described above, the regulation of energy metabolism through decreased mitochondrial electron transport chain functionality and consequent increased oxidative stress damage to lipids, proteins, and DNA, as well as calcium metabolism, may be central to the pathophysiology of BD (Fig. 1). Oxidative stress can cause structural modifications to DNA purine and pyrimidine bases or induce posttranslational

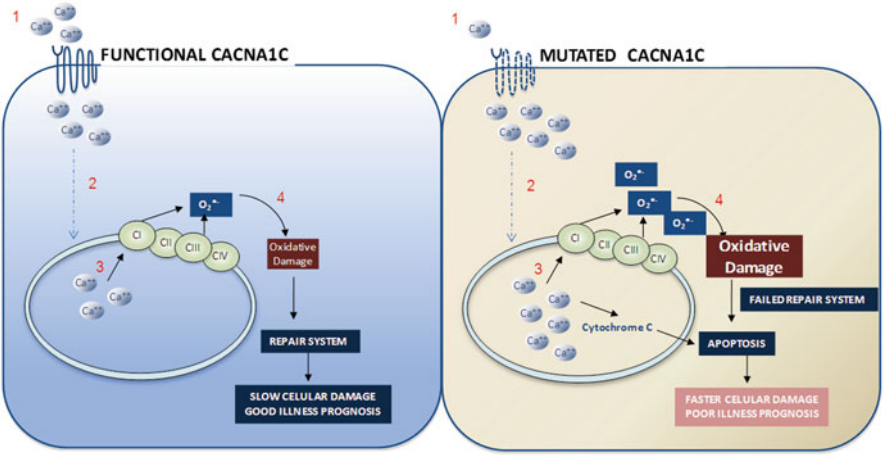

Fig. 1 Oxidative stress, mitochondrial dysfunction, and calcium voltage-dependent channels: an integrative model for bipolar disorder. *1*. Calcium influx is controlled by CACNA1C. When it is mutated, CACNA1C loses its ability to control Ca^{2+} influx. *2*. Protein interactors of CACNA1C will guide calcium signaling. *3*. Mitochondria can sense increased levels of Ca^{2+} to buffer it. Ca^{2+} is essential for normal mitochondrial electron transport chain (mETC) functionality. Under normal physiology, the leakage of superoxide ($O_2^{\bullet -}$) is controlled by the antioxidant repair system and the oxidative damage to biomolecules is under control. Excessive intra-mitochondrial Ca^{2+} concentration over-activates mETC, producing an excessive $O_2^{\bullet -}$. *4*. Combining excessive free radical production with failed antioxidant repair system, the oxidative damage to biomolecules will happen and it will affect protein functionality, leading to apoptosis if not stopped by antioxidant enzymes or repair system

modifications in proteins. Interestingly, epigenetic changes also play an important role in the etiology of BD. Epigenetics is characterized as a process that modifies gene expression through alteration in DNA methylation and chromatin structure without changing the genomic DNA sequence (Tseng et al. 2008). Using an epigenome-wide approach to verify DNA methylation of specific genes, Cui et al. (2007) found epigenetic differences at genes involved in neuronal development and loci implicated in oxidative stress and mitochondrial dysfunction. Recently, Nohesara et al. (2011) found that similar to their previous findings in the prefrontal cortex, MB-COMT promoter was hypomethylated (~50 %) in DNA derived from the saliva in SCZ and BD, compared to controls.

Emerging evidence suggests an interaction between oxidative stress and DNA methylation (Yucel et al. 2008). Cytosine (i.e. 5-hydroxymethylcytosine) or guanine oxidation (i.e. 8-hydroxy-2'-deoxyguanosine) promotes DNA demethylation through decreasing the affinity of the methyl group binding to DNA CpG islands, thus inducing changes in the expression of several genes (Fig. 2). Therefore, future studies evaluating the connection between oxidative damage to DNA and DNA methylation are essential to explain whether the oxidative stress can play a role in the well know decreased gene expression in BD (Wang et al. 2009).

Our understanding of molecular defects leading to BD is limited, which significantly prevents the development of new treatments for this illness. The evolution of

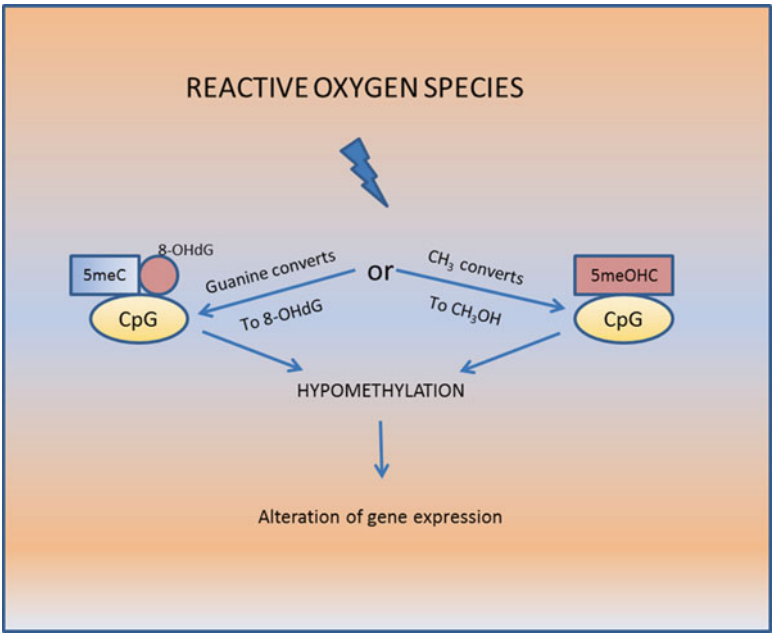

Fig. 2 Oxidative stress and DNA methylation/demethylation pathways. Reactive oxygen species (ROS); 5-meC, 5-methylcytosine; 5-meOHC, 5-hydroxymethylcytosine; 8-OHdG, 8-hydroxy-deoxyguanosine. Reactive oxygen species (ROS) induce redox alterations to guanine (8-OHdG) or cytosine (5-meC/5-meOHC), leading to DNA aberrations and alterations to gene expression

studies exploring the relationship among oxidative stress, DNA methylation, and gene expression will ultimately open avenues to the development of new strategies that may prevent oxidative stress damage to biomolecules, thus translating the knowledge from the bench to the clinic.

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