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ARTICLE

Number of manic episodes is associated with elevated DNA oxidation in bipolar I disorder

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Abstract

Bipolar disorder (BD) is a major public health problem characterized by progressive functional impairment. A number of clinical variables have been associated with progression of the disease, most notably number of affective episodes and presence of psychotic symptoms, both of which correlate with greater cognitive impairment, lower response rates for lithium, and possibly lower levels of neurotrophic factors. Oxidative damage to cytosine and guanosine (8-OHdG) has been described as a modulator of DNA methylation, but the extent of DNA oxidative damage involvement in BD remains unclear. The aim of this study was to evaluate the extent of DNA oxidative damage to 8-OHdG and 5-methylcytosine (5-HMec), as well as global methylation (5-Mec), in BD patients and healthy controls. Potential association with clinical variables was also investigated. DNA levels of 8-OHdG, 5-HMec and 5-Mec were measured in 50 BD type I patients and 50 healthy controls. DNA 8-OHdG levels were higher in BD patients compared to healthy controls and found to be positively influenced by number of previous manic episodes. BD subjects had lower levels of 5-HMec compared to controls, whereas this measure was not influenced by the clinical features of BD. Number of manic episodes was correlated with higher levels of 8-OHdG, but not of 5-Mec or 5-HMec. Lower demethylation activity (5-HMec) but no difference in global 5-Mec levels was observed in BD. This finding suggests that oxidative damage to 8-OHdG might be a potential marker of disease progression, although further prospective cross-sectional studies to confirm neuroprogression in BD are warranted.

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Introduction

Bipolar disorder (BD) is a major public health problem characterized by significant functional impairment and clinical co-morbidities (Wachsman, 1997; Kupfer, 2005). Although there are currently scant prospective longitudinal studies (mostly short duration) in BD patients demonstrating progressive dysfunction during the disease course, a number of clinical variables have been associated with disease progression in some cross-sectional studies (Swann et al., 2000; Robinson and Ferrier, 2006; Berk, 2009; Kauer-Sant'Anna et al., 2009). These variables include number of affective episodes and presence of psychotic symptoms, both of which have been correlated with greater cognitive impairment, elevated levels of inflammatory markers, lower neurotrophic factors, brain atrophy and poorer response to treatment (Swann et al., 2000; Robinson and Ferrier, 2006; Berk, 2009; Kauer-Sant'Anna et al., 2009). Despite recent advances, the molecular mechanisms underlying hypothetical neuroprogression remain unclear. However, cumulative evidence has shown that increased oxidative stress damage to biomolecules may be central to the pathophysiology of BD (Andreazza et al., 2008; Berk, 2009). Therefore maintaining the chemical integrity of DNA during assault by oxidizing agents represents a constant challenge in BD patients. In addition, the impact of features of the disease on DNA oxidation, such as number of affective episodes or psychotic symptoms, remains unclear.

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and hydroxyl radicals are produced

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as by-products of mitochondrial phosphorylation (Gutteridge and Halliwell, 2000; Cavanagh et al., 2002; Clark et al., 2002; Ferrier and Thompson, 2002). When mitochondrial and cytoplasmic enzymatic and non-enzymatic antioxidant systems are overwhelmed by elevated ROS levels, oxidative damage to DNA, lipids and proteins can ensue (Lenaz, 2001; Bora et al., 2010). Oxidative stress leads to multiple forms of DNA damage including base modifications, deletions, strand breakage and chromosomal rearrangements (Valko et al., 2004, 2006). Such damage to DNA has been shown to affect its ability to function as a substrate for DNA methyl transferases, resulting in global hypomethylation (Wachsman, 1997; Kupfer, 2005). ROS production is associated with increased DNA damage and chromosomal degradation and also with alterations in both hypermethylation and hypomethylation of DNA (Swann et al., 2000; Robinson and Ferrier, 2006; Lim et al., 2008; Berk, 2009; Kauer-Sant'Anna et al., 2009). More specifically, ROS react with guanosine DNA residues, leading to the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG; Swann et al., 2000; Coryell et al., 2001; Robinson and Ferrier, 2006; Berk, 2009; Kauer-Sant'Anna et al., 2009; Guo et al., 2011). 8-OHdG is a DNA adduct resulting from damage to DNA via ROS (Tohen et al., 1990; Kohen and Nyska, 2002; Klaunig and Kamendulis, 2004; Andreazza et al., 2008; Berk, 2009). Guanosine base is known to be the most susceptible to oxidative damage (Spassky and Angelov, 1997; Gutteridge and Halliwell, 2000; Cavanagh et al., 2002; Clark et al., 2002; Ferrier and Thompson, 2002; Altieri et al., 2008; Radak and Boldogh, 2010; Kryston et al., 2011). Recently, greater research attention has been dedicated to oxidation of cytosine residues in DNA, given that cystosines are the anchor for methyl groups in DNA (Lenaz, 2001; Bora et al., 2010; Branco et al., 2012). Determination of 5-methylcytosine (5-Mec) levels is a method for assessing global methylation; therefore hydroxylation of 5-Mec to 5-hydroxymethylcytosine (5-HMec) has become an important novel epigenetic marker (Valko et al., 2004, 2006; Guo et al., 2011). The role of ROS in the modulation of 5-HMec, however, remains unknown.

In the present study, we therefore investigated whether levels of DNA oxidative damage (8-OHdG), 5-Mec and 5-HMec, were altered in young BD type I subjects compared to healthy controls. The impact of number of affective episodes, disease duration and psychotic symptoms on DNA oxidation and methylation levels were also evaluated since these may reflect disease severity or progression.

Materials and method

A total of 50 symptomatic subjects (33 women) with BD type I (26 in mania and 24 in depressive episode), aged 18–40 yr, were included. Diagnoses were determined by psychiatrists using the Structured Clinical Interview (SCID-I; First et al., 1996) for DSM-IV-TR (DSM-IV, 2000). The patients included in this study were recruited from the 'LICAVAL clinical trial', designed to compare the efficacy of valproate + lithium *vs.* carbamazepine + lithium in treating BD type I (Campos et al., 2010). These patients were evaluated immediately after the wash-out period ($\geqslant$4 wk for antidepressants, mood stabilizers or antipsychotics, or $\geqslant$8 wk for depot medications) and prior to commencing use of medications. The Young Mania Rating Scale (Young et al., 1978) and Montgomery–Asberg Depression Rating Scale (Montgomery and Asberg, 1979) were used to evaluate severity of symptoms. Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, current substance abuse or that had undergone electroconvulsive therapy in the preceding 6 months, were excluded. As an indicator of illness severity (Berk et al., 2011), data on disease duration (age at first use of medication for mood symptoms), number of previous depressive and manic episodes and lifetime psychotic symptoms (hallucinations or delusions during mood episodes) were extracted from the SCID-I results. Subjects who proved unable to furnish sufficient information were excluded.

In the control group, 50 healthy volunteers (25 women) aged 18–40 yr were recruited at the University of São Paulo. All controls had no current or past history of psychiatric disorder according to the evaluation conducted by trained psychiatrists using the Mini International Neuropsychiatric Interview (Sheehan et al., 1998). Similarly, control subjects had no family history (first degree relatives) of mood or psychotic disorders and had no recent treatment or substance abuse over the previous 3 months.

The research ethics board of the Hospital das Clínicas of the University of São Paulo approved the study. Written informed consent was obtained from all study participants.

DNA oxidation and methylation

DNA blocks were collected using EDTA-coated tubes from whole blood according to the salting-out protocol (Laitinen et al., 1994). Oxidation to DNA was evaluated based on oxidation to guanosine, by assessing 8-OHdG levels, and to cytosine by measuring 5-HMec levels. The two markers were measured using competitive ELISA analysis kits from Stress Marq Biosciences Inc. (Canada) and Epigentek Group Inc. (USA), respectively (Shen et al., 2007). In order to evaluate whether oxidative stress through DNA oxidation induced lower levels of DNA methylation, global DNA methylation (5-Mec) was evaluated using an ELISA-based method (Tahiliani et al., 2009).

Statistical analyses

Subjects were classified into two groups (BD patients and healthy controls). The χ^2 test was used for categorical

Table 1. Sociodemographic and clinical characteristics of the sample

	Bipolar disorder ($n=50$)		Healthy controls ($n=50$)		
	Mean	S.D.	Mean	S.D.	p
Age	26.8	4.5	26.0	4.00	<0.32[a]
Gender (female/male)	33/17		25/25		0.08[b]
8-OHdG (pg/ml)	77.4	9.7	64.6	12.2	
5-HMec (ng/μl)	0.06	0.03	0.10	0.07	
5-Mec (ng/μl)	2.42	1.15	2.90	1.28	
Illness duration (yr)	5.0	3.7			
YMRS	14.06	8.3			
MADRS	20.08	9.04			
Number of manic episodes	4.04	2.12			
Number of depressive episodes	3.72	1.21			
Lifetime psychotic symptoms	50.00%				

8-OHdG, 8-Hydroxy-2′-deoxyguanosine; 5-Mec, 5-methylcytosine; 5-HMec, 5-hydroxymethylcytosine; YMRS, Young Mania Rating Scale; MADRS, Montgomery–Asberg Depression Rating Scale.
[a] t test.
[b] χ^2.
Significant level $p<0.05$.

Table 2. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), 5-methylcytosine (5-Mec) and 5-hydroxymethylcytosine (5-HMec) levels were entered as dependent variables in a multivariate analysis of covariance model using gender, age and group as covariates

Dependent variable	Parameter	B	d.f.	F	p	Partial η^2 (%)	Observed power (%)
8-OHdG	Group	13.36	1	35.3	<0.001	27.1	100.0
	Gender	−4.21	1	3.4	0.07	3.5	44.8
	Age	0.01	1	0	0.98	0.0	5.0
5-HMec	Group	−0.03	1	7.33	0.01	7.2	76.4
	Gender	−0.03	1	4.6	0.03	4.7	56.9
	Age	0	1	1.21	0.27	1.3	19.3
5-Mec	Group	−0.49	1	3.66	0.06	3.7	47.4
	Gender	0.05	1	0.04	0.84	0.0	5.5
	Age	0	1	0	0.95	0.0	5.0

Significant level $p<0.05$.

data and Student's t test for continuous data. First, concentrations of 8-OHdG, 5-Mec and 5-HMec were entered as dependent variables to a multivariate analysis of covariance (MANCOVA) model using age, gender and group as covariates. Subsequently, for the BD group only, 8-OHdG, 5-Mec and 5-HMec were input as dependent variables to a MANCOVA model using age, disease duration, number of manic episodes, number of depressive episodes as covariates, and current mood state, gender and lifetime psychotic symptoms as fixed factors. Finally, Pearson's correlation test was employed to investigate the influence of clinical variables on biochemical measures and to determine correlation among measures. Version 19.0 of the PASW statistics software package (SPSS Inc., USA) was used for all analyses.

Results

Sociodemographics and clinical characteristics of the sample are shown in Table 1. Groups did not differ according to gender or age characteristics.

The BD group presented higher 8-OHdG concentrations ($F=35.3$, d.f. $=1$, $B=13.3$, $p<0.001$) and lower 5-HMec concentrations ($F=7.3$, d.f. $=1$, $B=-0.03$, $p=0.008$) compared to the control group (Table 2; Fig. 1).

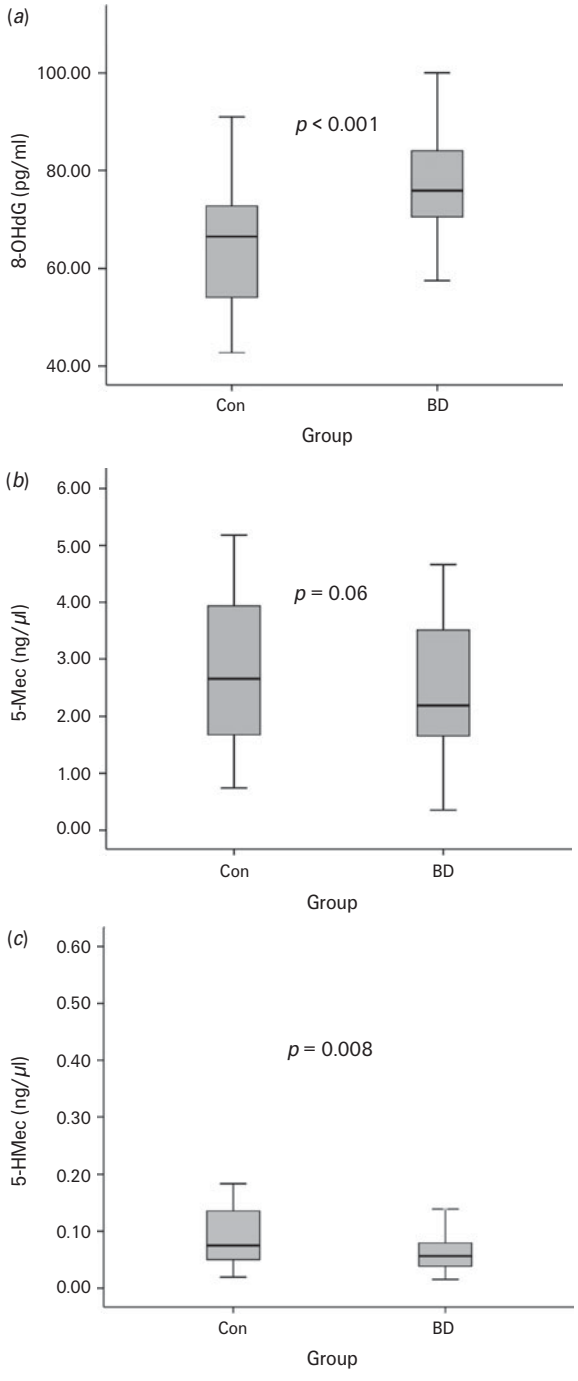

Fig. 1. Box plot graphic demonstrating simple comparison of mean levels and S.D. of (*a*) 8-hydroxy-2′-deoxyguanosine (8-OHdG), (*b*) 5-methylcytosine (5-Mec) and (*c*) 5-hydroxymethylcytosine (5-HMec) levels between healthy controls (Con) and bipolar disorder (BD) group.

Levels of 8-OHdG, 5-Mec and 5-HMec were not influenced by mood episode (manic or depressive; Table 3). In BD subjects, 8-OHdG levels were associated with higher number of past manic episodes ($F = 14.2$, d.f. $= 1$, $B = 2.2$, $p < 0.001$; Table 3). Disease duration showed a tendency to influence 8-OHdG concentrations ($F = 4.06$, d.f. $= 1$, $B = 0.66$, $p = 0.05$). Levels of 5-Mec and 5-HMec were not

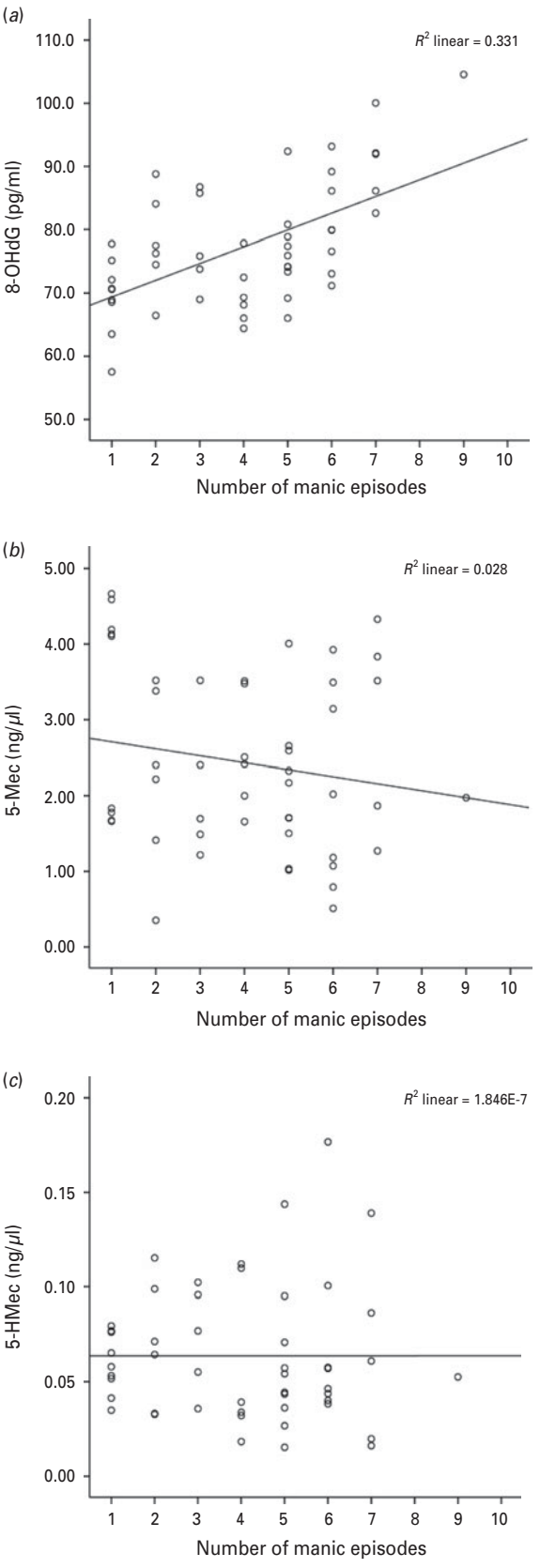

Fig. 2. Graphic demonstrating the correlation between number of manic episodes and (*a*) 8-hydroxy-2′-deoxyguanosine (8-OHdG; $p < 0.001$), (*b*) 5-methylcytosine (5-Mec; $p = 0.2$) and (*c*) 5-hydroxymethylcytosine (5-HMec; $p = 0.9$) levels.

Table 3. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), 5-methylcytosine (5-Mec) and 5-hydroxymethylcytosine (5-HMec) levels were entered as dependent variables in a multivariate analysis of covariance model using gender, age, mood state, illness duration, number of manic episodes, number of depressive episodes and lifetime psychotic symptoms as covariates in the bipolar disorder group

Dependent variable	Covariate	*F*	d.f.	*t*	*p*	Partial η^2 (%)	Observed power (%)
8-OHdG	Gender	0.33	1	0.58	0.56	0.8	8.8
	Age	0	1	−0.01	0.99	0.0	5.0
	Mood episode	0.01	1	−0.12	0.91	0.0	5.2
	Illness duration	4.06	1	2.02	0.05	8.8	50.4
	Number of manic episodes	14.2	1	3.78	<0.001	25.4	95.8
	Number of depressive episodes	0.02	1	0.16	0.88	0.1	5.3
	Lifetime psychotic symptoms	4.08	1	−2.02	0.05	8.9	50.6
5-Mec	Gender	3.9	1	−1.98	0.06	8.5	48.9
	Age	0.3	1	0.55	0.59	0.7	8.3
	Mood episode	0.26	1	0.52	0.61	0.6	8.0
	Illness duration	0.08	1	0.29	0.78	0.2	5.9
	Number of manic episodes	2.5	1	−1.59	0.12	5.6	34.1
	Number of depressive episodes	0.2	1	0.45	0.66	0.5	7.2
	Lifetime psychotic symptoms	0.45	1	0.21	0.83	0.1	5.5
5-HMec	Gender	0.43	1	0.66	0.51	1.0	9.9
	Age	0	1	0.08	0.94	0.0	5.1
	Mood episode	0.35	1	0.6	0.56	0.8	9.0
	Illness duration	0.04	1	−0.2	0.84	0.1	5.4
	Number of manic episodes	0.04	1	0.22	0.83	0.1	5.5
	Number of depressive episodes	0.15	1	−0.39	0.7	0.4	6.7
	Lifetime psychotic symptoms	0.59	1	0.77	0.44	1.4	11.8

Significant level $p<0.05$.

influenced by any of the clinical variables (Table 3). A positive correlation between number of manic episodes and 8-OHdG levels was also identified ($R^2=0.57$, $p<0.001$) which remained significant even after controlling for disease duration (Fig. 2). No correlation was observed for other biochemical measures and lifetime number of depressive symptoms or disease duration.

Discussion

To the best of our knowledge, this is the first study to show increased levels of 8-OHdG in patients with BD compared to healthy controls. Our results indicated that levels of 8-OHdG, but not 5-HMec, were positively related with number of manic episodes. However, no influence of current mood state on 8-OHdG concentration was found in unmedicated subjects with BD. These results suggest that oxidative damage to guanosine, but not cytosine, might be associated with disease severity or represent a measure reflecting cumulative mood episodes over the course of the disease, rather than a state-dependent marker.

Previous studies have reported high levels of 8-OHdG in depressive disorder (Forlenza and Miller, 2006; Kupper et al., 2009; Maes et al., 2009; Wei et al., 2009) and to date, this is the first study reporting the same finding in BD. Our results suggest that 8-OHdG levels are positively related with the number of manic episodes in patients with BD (Fig. 2). In support of this theory, other studies have reported that greater progressive deterioration in treatment response, cognitive function and brain morphology are associated with a higher number of mood episodes (Swann et al., 1997, 2000; El-Badri et al., 2001; Strakowski et al., 2002; Lyoo et al., 2006; Robinson and Ferrier, 2006). The potential association between lifetime number of mood episodes and DNA damage was investigated since mood episodes were previously shown to be associated with reduced response to lithium in BD (Swann et al., 1999, 2000). Cognitive deficits (verbal memory and executive dysfunctions) have also been shown to be associated with number of manic and depressive episodes in patients with BD (El-Badri et al., 2001; Robinson and Ferrier, 2006). In the same context, larger ventricular volume and lower cortical thickness have been described in BD subjects with longer illness duration and multiple episodes (Strakowski et al., 2002; Lyoo et al., 2006). However, more prospective studies with larger sample sizes are needed to fully appreciate the progressive nature of this devastating illness.

An emerging body of data also points to accelerated ageing as a consequence of a high number of mood episodes or long duration of illness (McEwen, 2003;

Kapczinski et al., 2008; Juster et al., 2010). Additionally, depressive symptoms have been associated with elevated rates of ageing-related diseases such as cardiovascular disease and possibly cancer, even after adjusting for differences in health-related behaviours such as smoking and exercise (Penninx et al., 1998; Everson-Rose et al., 2004; Evans et al., 2005; Gump et al., 2005). Thus, our results showing correlation between DNA oxidation and number of manic episodes may further corroborate the associations observed between ageing and progression of BD in earlier studies. Moreover, it has been suggested that manic episodes contribute to persistent oxidative stress.

Accumulation of oxidative damage is thought to lead to neuronal cell death by apoptosis or as a consequence of aggregation of oxidized proteins, which may result in impairment of mood stabilizing mechanisms (Berk et al., 2011). This modification has been shown to be both cytotoxic and mutagenic (Wood et al., 1990; Chen et al., 1995; Choi et al., 1999; Klungland et al., 1999) and is followed by induction of the stress response as well as antioxidant and DNA repair enzymes (Lenaz, 2001; Bora et al., 2010). Recent studies have shown evidence of DNA damage in BD, findings corroborated by our results (Andreazza et al., 2007; Buttner et al., 2007; Mustak et al., 2010). Buttner et al. (2007) reported higher DNA fragmentation in post-mortem anterior cingulate cortex of BD patients (Buttner et al., 2007). Mustak et al. (2010) indicated that both single and double strand breakages were high in post-mortem brain tissues of BD patients who died from suicide compared with controls (Mustak et al., 2010). Andreazza et al. (2007) reported that higher DNA damage (comet assay) appeared to be associated with severity of manic or depressive episodes, suggesting that the high oxidative stress found in BD may be responsible for elevated DNA damage (Andreazza et al., 2007).

To date, only one published study has addressed peripheral global methylation levels in BD, reporting that leucocyte global DNA methylation did not differ between BD and controls (Bromberg et al., 2009). Other authors have studied methylation levels in specific promoter regions with mixed results (Abdolmaleky et al., 2006; Kuratomi et al., 2008; Rosa et al., 2008; Dempster et al., 2011; Nohesara et al., 2011). In the present study, no difference in 5-Mec was found between patient and control groups, but lower levels of 5-HMec were observed in BD, perhaps indicating lower active DNA demethylation activity. Conversely, as shown in Fig. 1, there is a total overlap between BD and controls for distribution of 5-Mec and 5-HMec, suggesting a lack of sensitivity or specificity for BD.

Limitations of this study include the fact that the analysis of DNA oxidation and methylation was based on peripheral blood. To date, there are no data associating the status of peripheral DNA with that of brain tissue DNA regarding DNA methylation or gene expression (Shen et al., 2001; Plume et al., 2012). Oxidative damage to DNA can be induced by several factors other than the pathophysiology of the illness. These factors were reviewed in the study by Gutteridge and Halliwell (2010) and include exposure to excessive UV radiation, pesticides and environmental toxins. It is important to note that control cases would be equally exposed to the same environmental factors, strengthening the case for attributing the results to the pathophysiology of the disorder. Another limitation is the study design (cross-sectional) which may allow bias in memory regarding the number of mood episodes reported. Moreover, the fact that we studied only young BD subjects who agreed to enrol on a clinical trial should be considered a recruitment bias.

In summary, we demonstrated that a higher number of manic episodes were associated with increased DNA oxidative damage to guanosine. The present findings highlight a significant role of DNA oxidation, predominantly involving guanosine, in severe mood disorders. Further studies supporting this model that involve other biological targets associated with progressive course in BD are warranted. Moreover, further studies to clarify the relationship between central and peripheral DNA methylation and oxidation are also needed.

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Statement of Interest

None.

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