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Higher systemic oxidatively generated DNA and RNA damage in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives

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ABSTRACT

Background: Prior studies in bipolar disorders (BD) have suggested that oxidative stress and cellular ageing play a key role in the pathophysiology of BD. Nevertheless, oxidative stress has not been investigated in patients with newly diagnosed BD and in their unaffected first-degree relatives (UR), compared with healthy control individuals (HC).

Methods: We investigated the level of systemic oxidative damage to DNA and RNA measured by urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo) levels, respectively, in 360 patients with newly diagnosed BD, 92 of their UR and 197 HC.

Results: Independent of lifestyle and demographic variables, levels of both 8-oxoGuo and 8-oxodG was 17.1% (B = 1.171, 95%CI = 1.125–1.219, $p < 0.001$) and 21.2% (B = 1.212, 95%CI = 1.145–1.283, $p < 0.001$) higher, respectively, in patients with BD compared with HC and 13.3% (B = 1.133, 95%CI = 1.069–1.200, $p < 0.001$) and 26.6% (B = 1.266, 95%CI = 1.167–1.374, $p < 0.001$) higher, respectively, in UR compared with HC. Neither 8-oxoGuo nor 8-oxodG levels differed between patients with BD and UR. These findings were replicated in patients in full or partial remission and were consistent both in BD type I and II.

Conclusion: Overall, the findings of higher oxidative stress in patients with newly diagnosed BD and their UR suggest that systemic nucleoside damage by oxidative stress is present prior to onset and in the early stages of BD thereby potentially representing trait markers of BD.

1. Introduction

Bipolar disorder (BD) is a highly heritable [1], potentially progressive disorder with an increasing risk of manic and depressive episodes at decreasing intervals over time [2,3] and further associated with disability, cognitive impairment, decreased quality of life as well as a reduced life expectancy of 8–12 years [4]. The pathophysiology is still unclear, however, dysmetabolism [5,6] and cardiovascular diseases [7] are twice as common in patients with BD compared with the general population, adding to the reduced life expectancy in these patients [7–10]. Further, somatic disease occurs at significantly younger ages [7,10,11] in patients with BD compared with the general population in line with the theory of accelerated aging [12] and oxidative stress may possibly contribute to these epidemiological findings [13]. Oxidative stress is recognized as a major trigger for cardiovascular disease [14,15] and may possibly represent a common pathophysiological mechanism shared by dysmetabolic conditions, cardiovascular diseases and BD.

It has been hypothesized that cumulative inflammation and

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oxidative stress contribute to the pathophysiology of BD through increased generation of reactive oxidative species and DNA/RNA nucleoside damage causing telomere shortening and alterations in the electron transport chain in the mitochondria [16–19]. A validated method of determining systemic effects of oxidative damage is the measurement of the DNA damage marker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and RNA damage marker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoGuo) in urine by ultraperformance liquid chromatography with tandem mass spectrometry, which is considered a more reliable method than their measurements in blood samples [20,21]. This method measures oxidized nucleosides (guanine) that are excreted in urine independent of changes to RNA breakdown and DNA repair and neither 8-oxoGuo nor 8-oxodG seem influenced by diet [22,23]. Nevertheless, in vivo studies of urine 8-oxoGuo and 8-oxodG levels, which are indicative for RNA/DNA damage in patients with BD, are limited. A meta-analysis of oxidative stress, including 117 patients with BD and 113 healthy control individuals (HC) found higher oxidative stress measured as DNA/RNA damage in blood in patients with BD [24] in line with three subsequent studies from our group [25–27]. Thus, we have found increased urine 8-oxoGuo and 8-oxodG levels in 37 patients with rapid cycling BD compared with 40 HC[27] and in 54 patients hospitalized for acute mania compared with 35 HC[25]. Notably, in the study by Jacoby et al. oxidative stress levels were also increased during euthymia suggesting increased systemic nucleoside damage being a trait factor of BD [25]. Finally, cerebrospinal fluid nucleoside damage and urinary nucleoside damage are moderately correlated [28]. We recently showed that 8-oxoGuo measured in cerebrospinal fluid increased in patients with BD (n = 86) experiencing an affective episode between baseline and 12 months follow-up compared with HC (n = 44)[26] in line with the fact that oxidative stress seems to be associated with the number of previous manic episodes [29]. Nevertheless, these findings were contrasted by a study finding no difference in serum 8-hydroxy-2′-deoxyguanosine between 75 patients with BD and 60 HC [30]. Taken together, the majority of previous studies have found increased oxidative stress levels in small samples of patients with BD compared with HC. However, it is largely unknown whether oxidative stress levels are increased in patients with newly diagnosed BD and in their unaffected first-degree relatives (UR).

The aim of this study was to compare the urinary 8-oxoGuo and 8-oxodG levels in patients with newly diagnosed/first-episode BD, their UR, and HC without personal or first-degree history of affective disorders. Further, we aimed to determine to what extent illness and medication variables in patients with BD were associated with the urinary levels of 8-oxoGuo and 8-oxodG.

2. Materials and methods

2.1. Study design

The present study is a cross-sectional investigation of baseline data from the ongoing longitudinal Bipolar Illness Onset Study (BIO), which aims to identify composite biomarkers for BD in patients newly diagnosed with BD, their UR and HC. A full research protocol has been published for the BIO cohort study [31].

The study protocol has been approved by the Committee on Health Research Ethics of the Capital Region of Denmark (protocol No. H-7-2014-007) and the Danish Data Protection Agency, Capital Region of Copenhagen (RHP-2015-023). The study complies with the Declaration of Helsinki and its ethical principles.

2.2. Participants

2.2.1. Patients with bipolar disorder

Patients were recruited from the Copenhagen Affective Disorder Clinic. The Copenhagen Affective Disorder Clinic receives patients from the entire Capital Region of Denmark covering a catchment area of 1.6 million people as well as all psychiatric centers in the region and provides assessment and treatment service for patients with newly diagnosed/first episode bipolar disorder. All patients referred to the Copenhagen Affective Disorder Clinic as newly diagnosed/first episode BD, i.e. onset of first manic or hypomanic episode or when the diagnosis of BD is made for the first time, were routinely invited to participate in the BIO study. Inclusion criteria were an ICD-10 diagnosis of BD or a single manic or hypomanic episode and an age of 15–70 years. Patients were excluded if BD occurred secondary to a brain injury. The patients received treatment as usual while participating in our study.

2.2.2. Unaffected first-degree relatives

Upon obtaining consent from identified and recruited patients with BD, their first-degree relatives aged 15–70 (i.e. siblings and children) were invited to participate in the study. Relatives diagnosed with an ICD-10 psychiatric disorder below F34.0 were excluded from our study.

2.2.3. Healthy control persons

Age- and sex-matched healthy individuals, aged 15–70, without a personal or a first-degree family history of psychiatric disorders that had required treatment, were recruited on random days among blood donors from Danish Blood Bank at Rigshospitalet, Copenhagen, Denmark covering the same catchment area as patients with BD.

2.2.4. Diagnostics, data collection and clinical assessment

An initial diagnosis was made by medical doctors specialized in psychiatry according to the ICD-10 and DSM-IV criteria for type I and type II BD. After informed consent, medicine or psychology Ph.D. students verified the diagnosis using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) [32] categorizing patients into BD type I or type II. Clinical assessments of severity of depressive and manic symptoms were done using the Hamilton Depression Scale-17 items (HAM-D17) [33] and the Young Mania Rating Scale (YMRS) [34]. Medication usage was noted, the past months quality of sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI) [35] and activity level during the previous week was assessed using the International Physical Activity Questionnaire (IPAQ) [36]; moreover, BMI, educational level, daily alcohol intake and smoking habits were recorded. Lightly dressed and without shoes, height and weight were measured.

2.3. Laboratory methods

2.3.1. Urine collection and preparation

The participants were fasting from midnight until urine samples were collected the following day between 07.30 and 10.00 a.m. A freshly voided spot urine sample was obtained using a standard sampling kit without any additives (In Vitro, Fredensborg, Denmark). The sample was kept on ice and centrifuged at 4 °C and 1590 g for 15 min, after which aliquots of 1.5 ml were transferred to Eppendorf tubes and stored at −80 °C until analysis.

2.3.2. Urinary 8-oxodG and 8-oxoGuo levels

Urine samples were collected between June 2015 to April 2020 and analyzed in the Laboratory of Clinical Pharmacology, Rigshospitalet, Copenhagen, Denmark. The frozen samples were thawed, mixed and heated to 37 °C for 5 min then centrifuged at 10000 g for 5 min. The supernatants were assayed using ultraperformance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS). The chromatographic separation was performed on an Acquity UPLC system (Waters, Milford, MA, USA). The column used was an Acquity UPLC BEH Shield RP18 column (1.7 μm, 2.1 × 100 mm^2) protected with in-line filter (4 × 2 mm^2, 0.2 μm) both obtained from Waters. The MS detection of the nucleosides 8-oxoGuo and 8-oxodG was performed on an API 3000 triple quadrupole mass spectrometer (Sciex, Toronto, Canada) equipped with an ESI ion source (Turbospray) operated in positive mode. Further

details of the analysis are described elsewhere [22]. Creatinine concentrations were measured in the urine samples for oxidative stress levels to be divided by creatinine levels in accordance with Jaffe's reaction to adjust for the glomerular filtration rate [21].

2.3.3. Statistical analyses

Descriptive data were analyzed by chi-squared test for categorical data and by Student's t-test and Kruskal-Wallis test for continuous data according to whether assumptions of normal distribution were met or not. For analysis of continuous data, mixed effect regression models were further applied, accounting for familial relationship between relatives as a random effect and calculating the p-values between all three groups in the same model. Continuous data were presented as median and interquartile range when nonparametric and categorical data were presented as number and percentage.

First, we compared 8-oxoGuo and 8-oxodG levels, respectively, in unadjusted mixed effect regression models, with familial relationship as random effect to account for the correlation between family-related individuals. Second, for our main analyses, we compared 8-oxoGuo and 8-oxodG levels in mixed effect regression models adjusted for age and sex as independent variables. We repeated these models adjusted for age and sex, exclusively including patients in full or partial remission, defined as a score <14 on the HAM-D17 and < 14 on the YMRS, their UR and HC to investigate 8-oxoGuo and 8-oxodG levels as potential trait factors. Finally, we employed a fully adjusted model with sex, age, BMI, alcohol units per week, current smoking status (yes/no), HAMD-17, YMRS, PSQI (total score) and IPAQ (total score).

In multiple regression analyses among patients, we explored the association between 8-oxoGuo and 8-oxodG levels and medication and illness related variables. In these models, illness duration, current psychotropic medication in the form of antidepressants (yes/no), antiepileptics (yes/no), antipsychotics (yes/no) and lithium (yes/no) were entered as predictors along with age, sex, BMI, alcohol units per week, smoking (yes/no), HAMD-17 (total score), YMRS (total score), PSQI (total score) and IPAQ (total score) as covariates. Further, in similar models the four categorical psychotropic medication groups were substituted with the categorical variable receiving psychotropic medication (yes/no) and finally, illness duration was exchanged with number of affective episodes.

In post hoc analyses we compared 8-oxoGuo and 8-oxodG levels between the three groups (BD, UR and HC) in current non-smokers in models adjusted for sex and age. We also examined patients with BD type I and II, respectively, in the models with UR and HC adjusted for sex and age and further, analyzed the differences within patients between BD type I and II in models adjusted for sex and age. Subsequently, we examined 8-oxoGuo and 8-oxodG levels in patients with less than two years of untreated BD, UR and HC in models adjusted for sex and age.

The natural logarithm was applied to 8-oxodG and 8-oxoGuo if assumptions of normal distribution were not met. Results were presented as back transformed values with a parameter estimate, B, expressing the ration between increments in independent variables. All model assumptions were met. SPSS version 25 was used (SPSS for Windows Inc., Chicago, IL). The level of significance was set at $p < 0.05$.

3. Results

3.1. Demographic and clinical characteristics

We included 360 patients with newly diagnosed BD, 92 UR and 197 HC. Demographic and clinical characteristics of the study participants are presented in Table 1. Of the patients, 97.2%, were diagnosed with BD within the last two years. The three study groups showed no significant difference in sex or age distribution, except for UR being statistically significantly younger, see Table 1. Significantly more patients with BD were current smokers compared with UR (43.9% vs 21.1%, $p < 0.001$) and HC (43.9% vs 11.2%, $p < 0.001$). Notably, all three groups

Table 1
Demographic variables and oxidative stress levels in patients with newly diagnosed bipolar disorder (BD), their unaffected relatives (UR) and healthy controls persons (HC).

	BD	UR	HC	*P*-value
N	360	92	197	
Age (years)	29.0 [24.2–36.9]	26.7 [22.8–32.1]	27.6 [24.3–36.1]	0.946^{BD-HC} 0.011^{UR-HC} 0.003^{BD-UR}
Sex (% female)	234 (65.0)	54 (58.7)	127 (64.5)	0.991^{BD-HC} 0.608^{UR-HC} 0.501^{BD-UR}
Education (years total)	15 [13–17]	15 [13–17]	16 [15–17]	$<0.001^{BD-HC}$ 0.008^{UR-HC} 0.408^{BD-UR}
BMI (kg/m^2)	24.5 [22–27]	23.4 [21–27]	23.7 [22–26]	0.015^{BD-HC} 0.666^{UR-HC} 0.019^{BD-UR}
Number of smokers (%)	156 (43.9)	19 (21.1)	22 (11.2)	$<0.001^{BD-HC}$ 0.174^{UR-HC} $<0.001^{BD-UR}$
Alcohol (units per week)	2 [0–7]	2 [1–6]	5 [2–10]	0.006^{BD-HC} 0.023^{UR-HC} 0.704^{BD-UR}
HAMD-17	9 [5–15]	2 [0–4]	0 [0–2]	$<0.001^{BD-HC}$ 0.002^{UR-HC} $<0.001^{BD-UR}$
YMRS	3 [0–7]	0 [0–2]	0 [0–1]	$<0.001^{BD-HC}$ 0.735^{UR-HC} $<0.001^{BD-UR}$
IPAQ	1983 [1040–3685]	2400 [997–4845]	2798 [1538–4262]	0.167^{BD-HC} 0.989^{UR-HC} 0.287^{BD-UR}
PSQI	8 [6–11]	5 [3–7]	4 [3–5]	$<0.001^{BD-HC}$ 0.015^{UR-HC} $<0.001^{BD-UR}$
BD I	112 (31.1)	–	–	–
BD II	248 (68.9)			
Age of onset (years)	17 [14–21]	–	–	–
*Illness duration (years)	10 [6–16]	–	–	–
**Untreated bipolar disorder (years)	4 [1–10]	–	–	–
Affective episodes	12.5 [6–27]	–	–	–
Current affective state				
Remission	211 (58.9)	–	–	–
Mild/moderate depressive episode	86 (24)	–	–	–
Severe depressive episode	8 (2.3)	–	–	–
Manic episode	1 (0.3)			
Hypomanic episode	30 (8.4)	–	–	–
Mixed episode	20 (5.6)	–	–	–
N/A	2 (0.6)	–	–	–
Current psychotropic medication				
No psychotropic medication	61 (16.9)	–	–	–
Antidepressant treatment	47 (13.1)	–	–	–
Antipsychotic treatment	120 (33.3)	–	–	–
Antiepileptic treatment	187 (51.9)			
Lithium treatment	110 (30.6)	–	–	–

Continuous variables are presented as median [interquartile range]. Categorical variables are presented as n (%). Abbreviations: BMI: Body Mass Index; HAM-D-17: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; IPAQ:

International Physical Activity Questionnaires; PSQI: Pittsburgh Sleep Quality Index; N/A: Not applicable.

* Illness duration was defined as time from first episode (i.e. depressive, manic, hypomanic or mixed episode).

** Untreated bipolar disorder was defined as time from first manic, hypomanic or mixed episode to time of diagnosis.

had a median level of BMI within normal range, and the median was 24.5 [22–27] for patients with BD and statistically higher compared with UR and HC, respectively, see Table 1. Two patients with BD and one UR had diabetes type 1, one patient with BD had diabetes type 2 and five patients with BD, zero UR and one HC had a cardiovascular disease diagnosis.

3.2. Nucleoside damage from oxidative stress in patients with bipolar disorder, their unaffected first-degree relatives and healthy control persons

In unadjusted models the level of 8-oxoGuo was increased 17.1% in patients with BD compared with HC (BD vs. HC: B = 1.171, 95%CI = 1.124–1.220, p < 0.001) and 11.5% in UR compared with HC (UR vs. HC: B = 1.115, 95%CI = 1.052–1.182, p < 0.001), see Fig. 1. No significant difference was found between patients with BD and UR (BD vs. UR, B = 1.050, 95%CI = 0.996–1.107, p = 0.07). 8-oxodG levels were 21.3% higher in patients with BD (B = 1.213, 95%CI = 1.146–1.284, p < 0.001) and 25.9% higher in UR (B = 1.259, 95%CI = 1.161–1.366, p < 0.001) compared with HC, see Fig. 2. Patients with BD and UR had similar levels of 8-oxodG (B = 0.963, 95%CI = 0.895–1.037, p = 0.3).

In our main analyses adjusted for sex and age, 8-oxoGuo was 17.1% higher in patients with BD (B = 1.171, 95%CI = 1.125–1.219, p < 0.001) and 13.3% higher in UR (B = 1.133, 95%CI = 1.069–1.200, p < 0.001) compared with HC, see Table 2, Model 1. Levels of 8-oxodG was 21.2% higher in patients with BD (B = 1.212, 95%CI = 1.145–1.283, p < 0.001) and 26.6% higher in UR (B = 1.266, 95%CI = 1.167–1.374, p < 0.001) compared with HC, see Table 2, Model 1. When considering only patients in full or partial remission, 8-oxoGuo was 17.2% higher for patients with BD compared with HC (B = 1.172, 95%CI = 1.122–1.225, p < 0.001) and levels of 8-oxodG in patients with BD was 20.2% higher than HC (B = 1.202, 95%CI = 1.131–1.276, p < 0.001), whereas levels did not differ between patients with BD and UR (p = 0.2). Finally, excluding the 9 participants with a diagnosis of diabetes type I (n = 3), diabetes type 2 (n = 1) and/or cardiovascular disease (n = 5) from our main analyses did not alter results for 8-oxoGuo (BD vs. HC B = 1.170, 95%CI = 1.123–1.218, p < 0.001, UR vs. HC B = 1.137, 95%CI = 1.073–1.204, p < 0.001, BD vs. UR p = 0.3) or 8-oxodG (BD vs. HC B = 1.208, 95%CI = 1.141–1.278, p < 0.001, UR vs. HC B = 1.265, 95%CI = 1.166–1.372, p=<0.001, BD vs UR, p = 0.2).

In the fully adjusted models adjusted for sex, age, BMI, alcohol, smoking, HAMD-17, YMRS, IPAQ and PSQI: 8-oxoGuo was 15.5% higher in patients with BD (B = 1.155, 95%CI = 1.089–1.226, p < 0.001) and 13.4% higher in UR (B = 1.134, 95%CI = 1.067–1.206, p < 0.001) compared with HC, see Table 2, Model 2. Levels of 8-oxodG was 11.7% higher in patients with BD (B = 1.117, 95%CI = 1.029–1.214, p = 0.009) and 20.9% higher in UR (B = 1.020, 95%CI = 1.110–1.317, p < 0.001) compared with HC, see Table 2, Model 2.

3.3. Associations between illness duration, medication and oxidative stress levels in newly diagnosed patients with bipolar disorder

In analyses within patients, Table 3, lithium treatment was associated with 9.1% higher levels of 8-oxoGuo (B = 1.091, 95%CI = 1.026–1.60, p = 0.006) and 15.8% higher levels of 8-oxodG (B = 1.158, 95%CI = 1.062–1.262, p = 0.001), whereas antidepressants were associated with 10% lower levels of 8-oxodG (B = 0.900, 95%CI = 0.813–0.996, p = 0.042).

3.4. Post hoc explorative analyses

We repeated our main analyses adjusted for sex and age in current non-smokers and found patients with BD had 14.6% higher levels of 8-oxoGuo compared with HC (B = 1.146, 95%CI = 1.095–1.199, p < 0.001), and UR had 12.2% higher levels compared with HC (B = 1.122, 95%CI = 1.054–1.194, p < 0.001). Levels of 8-oxodG was 13% higher in patients with BD (B = 1.130, 95%CI = 1.060–1.204, p < 0.001) compared with HC and 26.3% higher in UR (B = 1.263, 95%CI = 1.158–1.379, p < 0.001) compared with HC.

In models adjusted for sex and age, levels of 8-oxoGuo (p = 0.6) and 8-oxodG (p = 0.4) did not differ between patients with BD type I and type II. Patients with BD type I showed 18.5% higher levels of 8-oxoGuo (B = 1.185, 95%CI = 1.122–1.252, p < 0.001) and 23.4% higher levels of 8-oxodG (B = 1.234, 95%CI = 1.146–1.329, p < 0.001) compared with HC. No significant difference was found between patients with BD type I and UR comparing levels of 8-oxoGuo (p = 0.2) or 8-oxodG (p = 0.5). Similarly, patients with BD type II showed 16.5% higher levels of 8-oxoGuo compared with HC (B = 1.165, 95%CI = 1.116–1.216, p < 0.001) and 20.3% higher levels of 8-oxodG compared with HC (B = 1.203, 95%CI = 1.132–1.279, p < 0.001). Comparing patients with BD

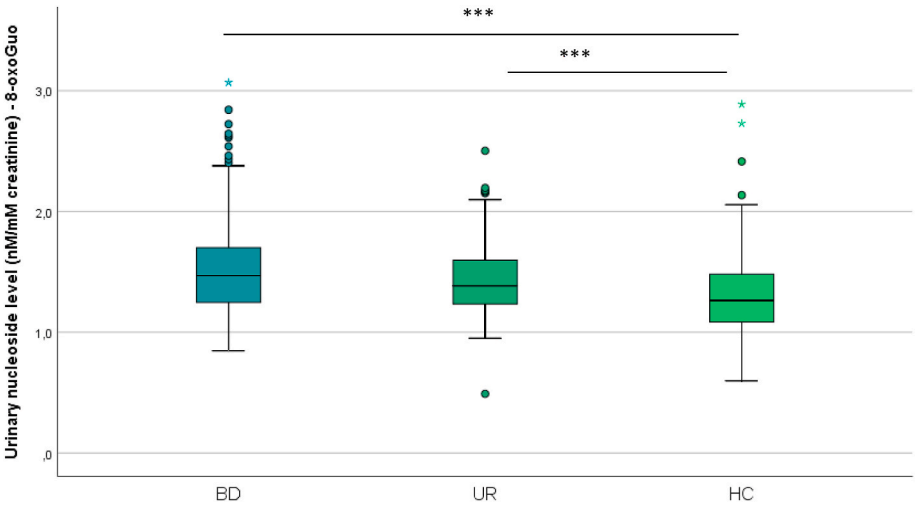

Fig. 1. Boxplot of oxidative stress marker 8-oxoGuo levels (nM/mM) in newly diagnosed patients with bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy control persons (HC). The lower and upper hinges represent the first and third quartiles. The upper and lower whiskers extend from the hinge to the largest and lower value, respectively. Data beyond the end of the whiskers are plotted individually. ***Statistical significance of p < 0.001.

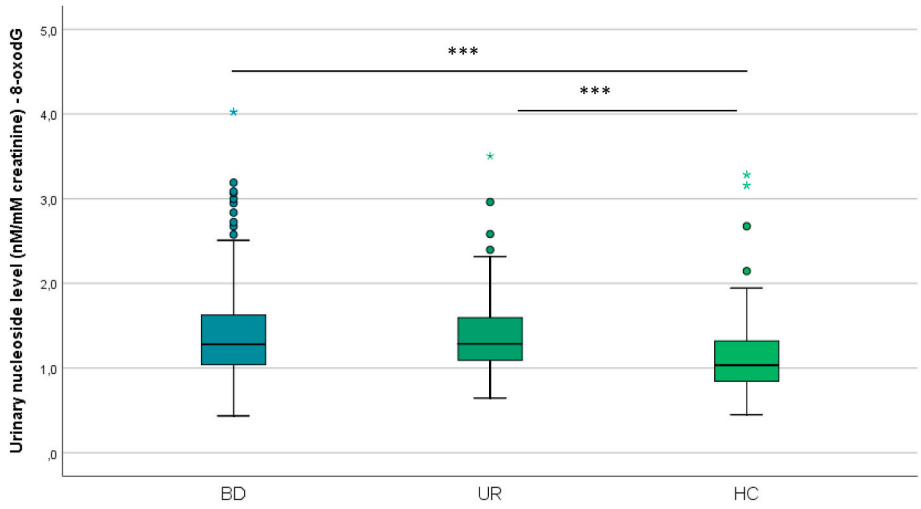

Fig. 2. Boxplot of oxidative stress marker 8-oxodG levels (nM/mM) in newly diagnosed patients with bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy control persons (HC). The lower and upper hinges represent the first and third quartiles. The upper and lower whiskers extend from the hinge to the largest and lower value, respectively. Data beyond the end of the whiskers are plotted individually. ***Statistical significance of $p < 0.001$.

type II with their UR, both 8-oxoGuo ($p = 0.4$) and 8-oxodG ($p = 0.2$) showed no statistically significant difference.

Finally, to verify our findings in patients with a duration of BD of less than two years ($n = 138$), we employed our main model, adjusted for sex and age, and found 16% higher levels of 8-oxoGuo ($B = 1.160$, 95%CI = 1.103–1.220, $p < 0.001$) and 20.7% higher levels of 8-oxodG ($B = 1.207$, 95%CI = 1.248–1.296, $p < 0.001$) compared with HC and no difference between patients with BD and UR.

4. Discussion

This is the first study on oxidative stress levels in patients with newly diagnosed BD and their UR compared with HC. The study profited from assessing reliable and validated measurements of systemic oxidative stress levels, 8-oxoGuo and 8-oxodG, in a large well characterized study population with a total of 649 participants comprising 360 patients with newly diagnosed BD, 92 of their UR and 197 HC. The levels of 8-oxoGuo and 8-oxodG were statistically significantly higher in both patients with BD and UR compared with HC in our main analyses adjusted for sex and age and further withstood adjustment for several possible predictors in fully adjusted analyses. To account for the possible state variation of oxidative stress, sub-analyses compared patients with BD in full or partial remission and found similarly higher 8-oxoGuo and 8-oxodG levels compared with HC. These findings were consistent both in BD type I and II. In analyses within patients with newly diagnosed BD, we found that lithium was associated with higher levels of oxidative stress, whereas antidepressants wereassociated with lower levels of oxidative stress.

4.1. Interpretation of findings in patients with BD

Our findings of increased levels of both the DNA and RNA oxidation marker in newly diagnosed patients with BD replicates previous findings from our group [25,27]. In line with Jacoby et al. [25], who exclusively studied BD type I, we also found higher 8-oxoGuo and 8-oxodG levels in full or partial remission and findings were consistent both in BD type I and II, supporting that 8-oxoGuo and 8-oxodG may represent trait markers in BD. In accordance with the two prior studies from our group [25,27], we did not find an association between illness duration or number of affective episodes and levels of oxidative stress.

The patients with BD in our study were newly diagnosed and 97% were diagnosed within the last two years. However, the median illness duration in this study was ten years and the median delay in diagnosis was four years in accordance with the well-known diagnostic delay of BD [37,38]. Therefore, in post hoc analyses, we examined the 138 patients with BD with less than two years of untreated BD to explore oxidative stress in early illness stages. Levels of both 8-oxoGuo and 8-oxodG were similarly higher as in our main analyses, supporting a key role of oxidative stress in early stages of BD.

Our data supports the American Heart Association's classification of BD in youth as a moderate risk factor for accelerated atherosclerosis and early cardiovascular disease [13] with putative pathophysiological mechanisms of increased oxidative stress and inflammation. Moreover, increased oxidative stress occurs across a number of both somatic and psychiatric illnesses, such as diabetes, cancer, schizophrenia and unipolar disorder [39,40] and hence is not specific to BD, but rather suggests a shared pathophysiology of these diseases. Notably, only few of our participants had a somatic comorbidity of either diabetes type 1 (two patients with BD and one UR) diabetes type 2 (one patient with BD) or cardiovascular disease (i.e. five patients with BD and one HC) and excluding these from analyses did not change our results.

Despite the putative antioxidant effect of lithium [41], similar to Knorr et al. [26] we found that treatment with lithium was associated with higher levels of oxidative stress and, similarly to Munkholm et al. antidepressants were associated with lower levels [27]. However, we used dichotomous treatment categories, thus results should be interpreted with caution as discussed in limitations.

4.2. Interpretation of findings in UR

In contrast to a recent twin-study from our group examining urinary 8-oxoGuo and 8-oxodG in remitted patients with affective disorders and their high-risk twins finding no difference between high-risk twins and HC [42], we found higher 8-oxoGuo and 8-oxodG in UR compared with HC, which suggests that oxidative stress might already be present prior to onset of BD. Our findings also contrast another small study of patients with BD ($n = 36$) and their siblings ($n = 39$) compared with HC ($n = 44$), that did not find differences in oxidative stress markers, however, they assessed less validated oxidative stress markers and methods (ELISA techniques) [17]. On the other hand, our results comport with the finding of shorter telomere lengths in both patients with BD and their UR in a small study by Vasconcelos-Moreno [17]. Interestingly, we have previously assessed the 30-year cardiovascular risk on a subsample of the included patients with BD, UR and HC and found a higher risk in both patients with BD and UR compared with HC [43], possibly explained by the higher levels of oxidative stress in line with a recent

Table 2
Levels of oxidative stress markers 8-oxoGuo and 8-oxodG in patients with newly diagnosed bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy control persons (HC).

Model		B	95%CI	*P*-value
1	8-oxoGuo			
	BD vs. HC	1.171	1.125–1.219	<0.001
	UR vs. HC	1.133	1.069–1.200	<0.001
	BD vs. UR	1.034	0.981–1.090	0.216
	Age	1.004	1.002–1.006	<0.001
	Male vs. female sex	0.949	0.913–0.985	0.007
	8-oxodG			
	BD vs. HC	1.212	1.145–1.283	<0.001
	UR vs. HC	1.266	1.167–1.374	<0.001
	BD vs. UR	0.957	0.889–1.031	0.248
	Age	1.000	0.998–1.003	0.801
	Male vs. female sex	0.920	0.872–0.971	0.002
2	8-oxoGuo			
	BD vs. HC	1.155	1.089–1.226	<0.001
	UR vs. HC	1.134	1.067–1.206	<0.001
	BD vs. UR	1.019	0.955–1.087	0.578
	Age	1.004	1.002–1.006	<0.001
	Male vs. female sex	0.947	0.909–0.986	0.009
	BMI	1.004	0.999–1.008	0.091
	Alcohol	0.996	0.993–0.999	0.020
	Smoking	1.040	0.996–1.087	0.078
	HAMD-17	1.000	0.997–1.004	0.835
	YMRS	0.999	0.994–1.004	0.753
	IPAQ	0.998	0.992–1.004	0.518
	PSQI	1.000	1.0000–1.000	0.983
	8-oxodG			
	BD vs. HC	1.117	1.029–1.214	0.009
	UR vs. HC	1.209	1.110–1.317	<0.001
	BD vs. UR	0.924	0.845–1.010	0.083
	Age	1.002	0.999–1.005	0.162
	Male vs. female sex	0.941	0.889–0.996	0.037
	BMI	0.990	0.983–0.996	0.001
	Alcohol	0.998	0.994–1.003	0.416
	Smoking	1.136	1.069–1.208	<0.001
	HAMD-17	1.007	1.001–1.012	0.015
	YMRS	0.997	0.989–1.004	0.353
	IPAQ	0.999	0.990–1.007	0.765
	PSQI	1.000	1.000–1.000	0.779

Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, BMI, alcohol, smoking, HAM-D17, YMRS, IPAQ and PSQI. Abbreviations: BMI: Body Mass Index; HAMD-17: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; IPAQ: International Physical Activity Questionnaires; PSQI: Pittsburgh Sleep Quality Index.

Table 3
Oxidative stress markers 8-oxoGuo and 8-oxodG in patients with newly diagnosed bipolar disorder.

Model		B	95%CI	*P*-value
1	8-oxoGuo			
	Age	1.003	1.000–1.007	0.082
	Male vs. female	1.071	1.014–1.132	0.015
	BMI	1.006	1.001–1.012	0.030
	Alcohol	0.996	0.993–1.000	0.070
	Smoking	1.057	1.004–1.112	0.034
	HAMD-17	0.993	0.939–1.050	0.809
	YMRS	1.013	0.908–1.130	0.820
	IPAQ	1.000	1.000–1.100	0.178
	PSQI	0.994	0.987–1001	0.082
	Illness duration	1.001	0.997–1.005	0.667
	Affective episodes*	1.000	1.000–1.001	0.411
	Antidepressants	1.021	0.949–1.098	0.574
	Antipsychotics	1.020	0.966–1.077	0.470
	Antiepileptics	0.992	0.940–1.048	0.776
	Lithium	1.091	1.026–1.160	0.006
	Receiving medicine**	0.978	0.914–1.046	0.513
	8-oxodG			
	Age	1.001	0.995–1.006	0.805
	Male vs. female	1.107	1.024–1.196	0.011
	BMI	0.989	0.981–0.997	0.005
	Alcohol	1.001	0.996–1.006	0.710
	Smoking	1.177	1.096–1.264	<0.001
	HAMD-17	0.937	0.866–1.013	0.103
	YMRS	0.976	0.837–1.137	0.751
	IPAQ	1.000	1.000–1.000	0.072
	PSQI	0.996	0.986–1.005	0.381
	Illness duration	1.001	0.995–1.006	0.831
	Affective episodes*	1.000	0.999–1.001	0.767
	Antidepressants	0.900	0.813–0.996	0.042
	Antipsychotics	0.983	0.911–1.060	0.649
	Antiepileptics	1.028	0.953–1.109	0.477
	Lithium	1.158	1.062–1.262	0.001
	Receiving medicine**	0.983	0.894–1.082	0.730

Model 1 adjusted for age, sex, BMI, alcohol, smoking, HAMD-17, YMRS, IPAQ and PSQI. Abbreviations: BMI: Body Mass Index; HAMD17: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; IPAQ: International Physical Activity Questionnaires; PSQI: Pittsburgh Sleep Quality Index.
* In a second model illness duration was substituted with affective episodes.
** In a third model the four categorical psychotropic medication variables were substituted with the categorical variable "Medication vs. medication free".

prospective study where high 8-oxoGuo levels were associated with cardiovascular mortality in patients with type 2 diabetes [15].

In line with findings of low heritability and high degree of environmental influence on DNA and RNA nucleoside damage [44], our findings could reflect underlying epigenetic alterations in patients with BD and UR growing up in potentially stressful environments in families having more mental illness and with a risk of exposure to childhood trauma, smoking, drug abuse and psychological and social stress [13,45].

Overall, our findings of higher oxidative stress in UR compared with HC could support oxidative stress being part of the pathogenesis of BD. However, final conclusions will rely on data from the ongoing longitudinal part of the BIO-study.

4.3. Possible predictors

Obesity has been associated with increased levels of oxidative stress [46,47]. In line with this, we found increasing BMI to be associated with higher levels of 8-oxoGuo in analyses within patients with BD. However, against expectations, in the analysis comparing patients with BD, UR and HC we found decreasing BMI to be associated with higher 8-oxodG. Notably, obesity was not a major issue in our cohort, in fact our three groups all had median BMI within the normal range. Prior studies have associated male sex with higher oxidative DNA damage in plasma [48] and in urine [49] corresponding with our findings within patients with BD. This is, however, in contrast with our findings of DNA and RNA damage being significantly higher in female sex compared with male sex in our analyses comparing the three groups. In accordance with the literature [50–52], smoking was also associated with elevated oxidative stress levels. To explore the influence of smoking, we repeated the analyses within non-smokers, exclusively, and still found higher oxidative stress levels in patients with BD and UR compared with HC. Thus, the well-described nucleoside damage from smoking [50–52] did not seem to drive our results. Furthermore, sleep plays a putative bidirectional role in oxidative stress levels [53,54], whilst physical exercise seems to increase oxidative stress [27,55], but neither PSQI total score nor IPAQ total score was associated with 8-oxoGuo or 8-oxodG. Finally, decreasing alcohol intake was statistically significantly associated with increasing 8-oxoGuo levels in comparison of the three groups, however, confidence intervals were very close to 1 (0.993–0.999), and this finding should be interpreted with caution, as discussed below.

4.4. Strengths and limitations

It is a strength that we included a large cohort of well-described patients with newly diagnosed BD, their UR and HC. Our study further profited from a high degree of standardization with urinary samples

being collected in a fasting state between 7.30 and 10 a.m. on the same day as participants were having a thorough clinical evaluation by a medical doctor or a psychologist trained in diagnosing BD.

However, some limitations apply to our study. First, our control group was recruited among blood donors without a personal or first-degree family history of psychiatric illness adding to the fact, that blood donors represent a super healthy population [56]. Nonetheless, the recruited blood donors were from the same catchment area as our patients with BD and matched with patients on sex and age. Education level was slightly higher in HC than in patients with BD and UR. However, it is possible that this reflects patients with BD being delayed in their studies due to their BD and in regard to UR possibly explained by the younger age. More patients with BD and UR were active smokers than HC. Nevertheless, results were still highly significant when adjusting for smoking as well as when excluding smokers. Alcohol intake was higher in HC than in patients with BD. This may, however, reflect that patients with newly diagnosed BD recently started in the Copenhagen Affective Disorder Clinic, where cessation is strongly recommended and supported. In the preceding months leading up to starting in the clinical program, many BD patients reported a substantially higher level of weekly alcohol intake exceeding the Danish Health Authority's recommended threshold. However, we only assessed alcohol intake based on the preceding month, therefore results regarding alcohol intake should be interpreted with caution. Alltogether, we consider our control group a pragmatic choice, as other ways to recruit HC such as via advertisements or national registers result in low response rates and likely selection bias.

Second, we used dichotomous treatment categories, which fail to capture the effects of dose and duration of treatment or overlap of treatments. Thus, our results regarding psychotropic medication should be interpreted with caution. Third, we did not adjust for oral contraception, which has been shown to elevate 8-oxoGuo and 8-oxodG in female sex [25,27] and could explain our unexpected finding of higher DNA and RNA damage in female sex compared with male sex in our analyses between the three groups. Finally, our measures of oxidative stress are global measures of oxidative stress and not tissue specific measures [23]. Nucleic acids and their precursors are only found in very small amounts extracellularly. The oxidation products therefore can be interpreted as a reflection of intracellular oxidative processes. This is at variance with other markers of oxidative stress, e.g. 3-nitrotyrosine and F2 isoprostanes that are intra-as well as extracellularly located, which reduces the specificity regarding origin. Even for 8-oxodG and 8-oxoGuo we have previously revealed differences, not only with regard to amount oxidized, but also concerning the prognostic value as only 8-oxoGuo is prognostic for death in type 2 diabetes [15]. The reason for this is not known, but we hypothesize it to be related to RNA being close to the mitochondria, therefore reflecting the mitochondria ROS production. The major disadvantage of using urine collection is that it reflects oxidation processes summarized for the entire body over 24 h. It is therefore insensitive in the detection of increased oxidative stress confined to single organs, particularly small organs, because of the "background" production in the remaining of the body. There is, however, no reason to assume that this is different for other markers, such as F2 isoprostanes or 3-nitrotyrosine, when measured in urine [57,58]. Accordingly, urinary markers are best suited to detect conditions where all or most of the body is exposed to increased oxidative stress, however, negative results should be interpreted with caution because of insensitivity to detect localized oxidative stress.

In conclusion, we found higher levels of systemic nucleoside damage in patients with newly diagnosed BD and their UR compared with HC. Our findings underline the presence of oxidative stress in UR and early stages of BD. These findings show that elevated 8-oxoGuo and 8-oxodG may represent trait markers of BD and could play an etiological role in the development of BD.

Declaration of competing interest

MV discloses within the last three years consultancy fees from Lundbeck, Sunovion and Janssen-Cilag. LVK, KC and SS have within the preceding three years been a consultant for Lundbeck.

The remaining authors declare no conflicts of interest.

The data that support the findings of this study are baseline investigations from the ongoing longitudinal BIO-study (conducted in 2015–2025), and will be available after cessation of the study. However, data may be available from the corresponding author upon reasonable request.

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