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Hair cortisol in newly diagnosed bipolar disorder and unaffected first-degree relatives

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ABSTRACT

Objective: Hair cortisol is a promising new biomarker of retrospective systemic cortisol concentration. In this study, we compared hair cortisol concentrations in patients with newly diagnosed bipolar disorder (BD), their unaffected first-degree relatives and healthy individuals and identified potential predictors of hair cortisol concentrations in patients with BD.

Method: In a cross-sectional design, we compared hair cortisol concentrations in 181 patients with newly diagnosed/first episode BD, 42 of their unaffected first-degree relatives and 101 healthy age- and sex-matched individuals with no personal or first-degree family history of affective disorder. In patients with BD, we further investigated whether medication- and illness related variables, as well as measures of stressful life events in the preceding 12 months and childhood trauma, were associated with hair cortisol concentrations.

Results: Hair cortisol concentrations were 35.1% (95%CI: 13.0–61.5) higher in patients with BD ($P = 0.001$) compared with healthy individuals in models adjusted for age and sex. Hair cortisol concentrations in unaffected first-degree relatives did not differ from healthy individuals ($P = 0.8$). In patients, neither medication, illness duration nor stress related variables were associated with hair cortisol concentrations.

Conclusion: We found elevated hair cortisol concentrations in patients newly diagnosed with BD indicating the presence of physiological stress in early stages of BD.

1. Introduction

Stressful life events often precede the first major mood episode of bipolar disorder (BD) (Horesh et al., 2011) as well as subsequent mood episodes (Lex et al., 2017) and the tolerance for stressful life events seems to decrease with advanced illness burden (Kessing and Andersen, 2017). Further, BD seems associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Belvederi Murri et al., 2016) including elevated concentrations of basal plasma and salivary cortisol concentrations although with large heterogeneity in findings (Belvederi Murri et al., 2016; Stalder and Kirschbaum, 2012). A major reason for the heterogeneous findings may be that plasma and saliva and also urine reflect circulating cortisol concentrations at the test moment that vary considerably with food intake, exercise, menstrual cycle, sleep and acute stress (Montero-Lopez et al., 2018; Stalder and Kirschbaum, 2012). In contrast, measurements of hair cortisol are indicators of systemic cortisol concentrations within the preceding months (Stalder and Kirschbaum, 2012), validly reflecting prolonged stress (Staufenbiel et al., 2013).

Assessment of hair cortisol concentration has several other benefits: first, it is a non-invasive procedure; second, the observed concentration is unaffected by sampling procedure and diurnal variations; and third, the measured concentration reflects an average concentration of free circulation cortisol in the months before the sample collection depending on the length of the hair sample (Russell et al., 2015; Stalder and Kirschbaum, 2012). A further advantage is that hair can be stored for years at room temperature while maintaining the same cortisol concentration over time (Russell et al., 2015). Toxicology and forensic science have integrated hair analysis for exogenous substances for more than 30 years (Pragst and Balikova, 2006) and international laboratories are collaborating toward standardization of hair cortisol measurements (Russell et al., 2015). Single hair cortisol concentration measurements include a strong trait component explaining 59–82% of the variance in contrast to state-related factors, which only explain a minor part of the variance (Stalder and Kirschbaum, 2012).

Only two studies have compared hair cortisol concentrations in

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patients with BD and healthy individuals. The first study did not find any difference in hair cortisol concentrations in 100 patients with BD and 195 healthy individuals, but found that patients with onset ≥30 years had elevated cortisol concentrations compared with patients with onset < 30 years in exploratory analyses (Manenschijn et al., 2012). The second study observed higher concentrations of hair cortisol in the 61 patients with BD type I compared with 82 healthy individuals (Streit et al., 2016). These two prior studies included patients with BD of an average age of 45–52 years (Manenschijn et al., 2012; Streit et al., 2016), hence patients with BD were supposedly in a later illness stage. Due to clinical progression of BD over time, it is relevant to study hair cortisol concentrations in earlier stages of BD and also in individuals predisposed to BD.

Higher salivary basal cortisol concentrations have been found in offspring of patients with BD than in offspring of individuals without mental illness (Ellenbogen et al., 2006, 2010). Another small prospective study of young offspring of patients with BD (n = 28) and offspring not predisposed to affective disorders (n = 31) found increased baseline salivary concentrations associated with onset of affective disorder within a 2.5-year follow-up period (Ellenbogen et al., 2011). In the present report, baseline hair cortisol concentrations were investigated. Moreover, in the same study, we currently collect hair cortisol samples from high-risk individuals over a 5-year period to investigate if hair cortisol may act as a risk predictor or later BD illness onset.

Hair cortisol concentrations have not previously been investigated in newly diagnosed patients with BD. Further, hair cortisol concentrations have neither been investigated in unaffected first-degree relatives of patients with BD.

1.1. Aim and hypotheses

The aims of the present study were: (I) to compare the cumulative hair cortisol concentrations over the preceding three months in patients with newly diagnosed/first episode BD and their unaffected first-degree relatives with healthy individuals. (II) to determine whether illness- and medication variables as well as measures of stressful life events and childhood trauma in patients with BD were associated with hair cortisol concentrations.

We hypothesized that concentrations of hair cortisol would be elevated in patients with BD and - to a lesser degree - in their unaffected first-degree relatives compared with healthy individuals without a family history of psychiatric disorders. Further, we hypothesized that higher hair cortisol concentrations would be associated with longer illness duration, stressful life events and childhood trauma.

2. Materials and methods

2.1. Study design

The recruitment for the current study took place from June 2015 to September 2017. The present report is a cross-sectional investigation of baseline data from the ongoing longitudinal Bipolar Illness Onset Study (BIO), which aims to identify biomarkers for BD (Kessing et al., 2017). The study protocol was 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). All participants provided written informed consent. The study complied with the Declaration of Helsinki principles (Seoul, October 2008).

2.2. Participants

2.2.1. Patients with bipolar disorder

Patients were recruited from the Copenhagen Affective Disorder Clinic that covers the Copenhagen catchment area of 1.6 million inhabitants (Region Hovedstaden) and offers service for patients newly diagnosed with BD. All patients referred to the Copenhagen Affective Disorder Clinic as newly diagnosed with BD or having a first episode of mania or hypomania were routinely invited to participate in the BIO study. Inclusion criteria were an ICD-10 diagnosis of BD or a single manic episode and age 15–70 years. Exclusion criterion was having an organic BD secondary to brain injury. Patients with BD received treatment as usual in the Copenhagen Affective Disorder Clinic without interference from study investigators.

2.2.2. Unaffected first-degree relatives

Siblings and children of the included patients with BD were invited to participate upon consent by the participating patient. Inclusion criteria were being a first-degree relative of an included patient with BD and aged 15–40 years. Exclusion criteria were an ICD-10 diagnosis lower than F34 including substance abuse, psychotic illnesses and a diagnosis of unipolar disorder or BD. We did not restrict the number of unaffected first-degree relatives included per patient with BD; however, we adjusted our analysis for the familial relationship.

2.2.3. Healthy individuals

Age- and sex matched healthy individuals were recruited among blood donors from the Blood Bank at Rigshospitalet, Copenhagen, Denmark by contacting blood donors in the waiting room at the Blood Bank on random days. Inclusion criterion was age 15–70 years. Exclusion criteria were a personal or first-degree family history of psychiatric disorders that had required treatment.

2.3. Clinical assessments

The initial diagnostic assessment of patients was held by a specialist in psychiatry, in the Copenhagen Affective Disorder Clinic, diagnosing patients with BD according to ICD-10 and classifying patients with BD as type I or type II according to DSM-5 criteria as part of daily practice. Following informed consent, the initial clinical diagnosis of BD was confirmed in a semi-structured research based interview using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990). Diagnosis of the current affective state was based on ICD-10 criteria. Severity of depressive and manic symptoms was assessed using the Hamilton Depression Rating Scale-17 items (HAMD-17) (Hamilton, 1960) and the Young Mania Rating Scale (YMRS) (Young et al., 1978), respectively. The Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1994), Stressful Life Events (SLE) (Kendler et al., 1998) and the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) were administered at the day of assessment. We used the PSQI global score, where a score > 5 indicates sleep disturbance (Buysse et al., 1989).

Absence of lifetime psychiatric morbidity defined by ICD-10 was confirmed for healthy individuals whereas psychiatric morbidity of F34 and higher according to ICD-10 were registered for unaffected first-degree relatives. Current medication was recorded for all participants.

2.4. Anthropometric assessment

After a 10-minutes rest blood pressure was measured using a calibrated automatic sphygmanometer (Microlife BP A3 plus). Lightly dressed and without shoes, height was measured to the nearest millimetre on a rigid stadiometer and weight was measured to the nearest 0.1 kg using a calibrated floor scale (Kern MPE PM). Waist circumference was measured as the midpoint between the lowest rib and the iliac crest in an upright position to the nearest millimetre as described in the World Health Organisations guidelines (Cornier et al., 2011).

2.5. Hair sample collection and analysis

2.5.1. Hair sample collection and storage

Hair samples were cut with fine scissors as close as possible to the scalp in the posterior vertex region, where hair growth rates are most uniform (Pragst and Balikova, 2006) and approximately grows one cm per month (Wennig, 2000). Notably, the most recent month seems not to be reflected in the hair samples, as the growth phase within the scalp is approximately two weeks and sampling with scissors unavoidably leaves a few millimeters of hair on the scalp, thus the proximal cm of the collected hair samples presumably was made at least a month prior (LeBeau et al., 2011). We used the proximal 3 cm of the hair as recommended by Stalder et al (2017), because hair cortisol concentrations are found to decline 29% from the proximal 3 cm segment to the following 3 cm segment (Stalder et al., 2017). During sampling hair samples were cut to approximately three cm in length and had a weight around 10 mg. The minimum weight was a 2.3 mg hair sample. Samples were stored for up to 24 months at room temperature in polypropylene microtubes and kept in flamingo boxes out of sunlight exposure.

2.5.2. Hair cortisol analysis

The hair samples were analysed by liquid chromatography and tandem mass spectrometry (LC–MS/MS) as described by Chen et al (2013) at Department of Environmental Medicine, University of Southern Denmark. In brief, the hair samples were carefully removed from the test tube with a tweezer, washed with methanol and dried at room temperature. Each hair sample was cut into 4–5 pieces into a tarred 2 mL polypropylene cryotube and weighed. An aliquot of 100 μL 20 ng/mL isotope labeled cortisol (cortisol-D4) (Sigma-Aldrich, St. Louis, MI) in methanol was added to the sample vial as internal standard, together with 0.9 mL of methanol. The samples were incubated while whirl mixed at 2000 RPM at 25 °C in the dark for a period of five days. After the 5 days of incubation, the samples were centrifuged at 3000 *g* for 5 min prior to 20 μL of the supernatant was transferred to the analytical system.

The utilized LC–MS/MS system consisted of a PAL Autosampler (CTC analytics, Zwingen, Switzerland), and an Accella 1250 Pump and a TSQ Quantiva Triple-Stage Quadrupole Mass Spectrometer (Thermo Scientific, San Jose, CA). The analytical column was a Kinetic C18 column, 100 x 4.6 mm (2.6 μm) (Phenomenex, Torrance, CA). The calibration curve and calculation of the sample concentration were based on the area ratio of the analyte/isotope labeled internal standard. Quality control samples were included in each series of samples analyzed. The limit of quantitation (LOQ) of the method was one pg/mg hair. The intra-day repeatability coefficient of variation was 8.7% and the inter-day reproducibility coefficient of variation was 9.5%.

Personnel performing the analysis of the samples were unaware of participation status of the samples.

2.6. Statistical analyses

Descriptive data were analysed by chi-square tests for categorical data and by Mann-Whitney U test for two independent groups for continuous data. Continuous data were presented as median and quartiles if assumptions of normal distribution were not met.

The analysis strategy was planned a priori and was based on prior identified predictors of cortisol concentrations (Belvederi Murri et al., 2016; Manenschijn et al., 2011; Stalder et al., 2017). For illustrative purposes, we first compared hair cortisol concentrations in an unadjusted linear mixed effect model with group (patient with BD, unaffected relative, healthy individual) as independent variable and familial relationship as random effect, to account for the correlation between family related individuals. Secondly, for our primary analyses, we employed a similar linear mixed effect model with hair cortisol concentration as the dependent variable and group (patient with BD, unaffected relative, healthy individual) as the independent variables, adding the covariates age and sex. Thirdly, in a fully adjusted model, we included age, sex, waist circumference, systolic blood pressure, childhood trauma (CTQ total score), stressful life events (SLE, 12 months score), YMRS (total score), HAMD-17 (total score) and sleep disturbances (PSQI total score).

In a multiple regression analysis among patients with BD, hair cortisol concentration was entered as the dependent variable, with age, sex, waist circumference, systolic blood pressure, childhood trauma (CTQ total score), stressful life events (SLE, 12 months score), YMRS (total score), HAMD-17 (total score), sleep disturbances (PSQI total score), illness duration, BD type (I or II), and current treatment with lithium (yes/no), antipsychotics (yes/no), antidepressants (yes/no) and antiepileptics (yes/no) as independent variables. We repeated the analysis, substituting the four treatment variables with the categorical variable 'receiving psychotropic medication' versus 'medication free'. Patients with a single manic episode (n = 3) were included in the BD type I group in these analyses according to the DSM-5 classification.

For all parametric tests, hair cortisol concentrations were transformed by the natural logarithm and subsequently all model assumptions were met. Results are presented as back transformed values with a parameter estimate B, expressing the ratio between increments in independent variables. The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS version 22.

3. Results

3.1. Demographic and clinical characteristics

We included 181 patients with BD, 42 of their unaffected first-degree relatives and 101 healthy individuals. Five patients with BD were Asian, five patients with BD were of mixed Asian and Caucasian ancestry and the remaining participants were Caucasian. Demographic and clinical variables of the study participants are presented in Table 1. The unaffected first-degree relatives were relatives of 36 patients with BD, as six patients with BD had two first-degree relatives included.

3.2. Hair cortisol concentration in patients with bipolar disorder, their unaffected first-degree relatives and healthy individuals

As listed in the top of Table 1 and depicted in the box plot, Fig. 1, the median [interquartile range] hair cortisol concentration was 8.9 [6.1–13.3] ng/mg hair in patients with BD, 6.6 [3.8–9.3] ng/mg hair in their unaffected first-degree relatives and 6.4 [4.5–9.6] ng/mg hair in healthy individuals. In the unadjusted mixed effect regression model, we found 33.6% higher hair cortisol concentration in patients with BD compared with healthy individuals (B = 1.336, 95%CI: 1.113–1.604, P = 0.002), whereas hair cortisol concentrations did not differ between unaffected first-degree relatives and healthy individuals (B = 0.915, 95%CI: 0.698–1.200, P = 0.5). In our main analysis, adjusted for group, sex and age, hair cortisol concentration was similarly elevated 35.1% in patients with BD ($B = 1.351$, 95%CI: 1.130–1.615, $P = 0.001$) but not higher in their first-degree relatives ($B = 0.970$, 95%CI: 0.742–1.268, $P = 0.8$) compared with healthy individuals. In this model, male sex and increased age were associated with increased hair cortisol concentrations (Table 2). In a similar model, adjusted for group, sex and age, hair cortisol concentration was 39.2% higher in patients with BD compared with their first-degree relatives ($B = 1.392$, 95%CI: 1.093–1.772, $P = 0.008$). Two patients were outliers with hair cortisol concentrations above 200 ng/mg hair. We did not have any good reason to exclude these patients from primary analyses; however, due to lack of extra hair we could not rerun the analysis and confirm accurate measurement of hair cortisol concentrations. To confirm validity of our findings we repeated our main analysis excluding participant with hair cortisol concentrations above 200 ng/mg hair. In this model, adjusted for group, sex and age, patients with BD had 29.2% higher hair cortisol concentrations ($B = 1.292$, 95%CI: 1.096–1.522, $P = 0.002$) compared

Table 1
Hair cortisol concentrations, demographic and clinical variables in patients with bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy individuals (HC).

	BD (1)	UR (2)	HC (3)	P-value
N	181	42	101	
Hair cortisol concentrations (pg per mg hair)	9.0 [6.1–13.3]	6.6 [3.8–9.3]	6.4 [4.5–9.6]	$< 0.001^{1-3}$ 0.7^{2-3}
Age (years)	29 [24–36]	26 [22–31]	28 [24–36]	0.6^{1-3} 0.014^{2-3}
Sex (% female)	121 (66.8)	25 (59.5)	65 (64.4)	0.7
Education (years total)	15 [12.5–16]	15.5 [13.5–17]	15.5 [14–17]	0.001^{1-3} 0.7^{2-3}
HAMD-17	9 [5–15]	2 [0–4]	1 [0–2]	$< 0.001^{1-3}$ 0.009^{2-3}
YMRS	2.5 [0–6]	0 [0–2]	1 [0–2]	$< 0.001^{1-3}$ 0.8^{2-3}
Waist circumference	85 [78–85]	79 [73–83]	81 [75–89]	0.011^{1-3} 0.2^{2-3}
Systolic blood pressure (mmHg)	119 [110–128]	124 [111–133]	123 [115–135]	0.005^{1-3} 0.6^{2-3}
Childhood trauma (CTQ total score)	37 [30–45.25]	30 [26–33]	27 [25–29]	$< 0.001^{1-3}$ $< 0.001^{2-3}$
Stressful life events (SLE 12 months score)	9 [5–12]	6 [3–8]	0 [0-0]	$< 0.001^{1-3}$ $< 0.001^{2-3}$
Sleep disturbances (PSQI total score)	10 [7–13]	6 [4–9]	4 [3–6]	$< 0.001^{1-3}$ 0.001^{2-3}
BD I	64 (35.4)			
BD II	114 (63.0)	–	–	
Single manic episode	3 (1.7)	–	–	
Age of onset	17 [14–21]	–	–	
Illness duration (years)*	10 [6–16.5]	–	–	
Untreated bipolar disorder (years)**	5 [1–11]			
Manic episodes	0 [0–1]	–	–	
Hypomanic episodes	4 [2–15]	–	–	
Depressive episodes	6 [3–15]	–	–	
Mixed episodes	0 [0–0]	–	–	
Total affective episodes	12 [6–29.5]	–	–	
Hospitalizations	0 [0–1]	–	–	
Current affective episode				
Remission	109 (60.2)	–	–	
Mild/ moderate depressive episode	37 (20.4)	–	–	
Severe depressive episode	4 (2.3)	–	–	
Mixed episode	8 (4.4)	–	–	
Hypomanic episode	14 (7.7)	–	–	
Manic episode	4 (2.2)	–	–	
N.A.	5 (2.7)	–	–	
Medication				
No psychotropic medication	24 (12.7)	–	–	
Lithium treatment	65 (35.7)	–	–	
Antiepileptic treatment	96 (53.0)	–	–	
Antidepressant treatment	45 (24.9)	–	–	
Antipsychotic treatment	56 (30.8)	–	–	

Continuous variables are presented as median [interquartile range]. Categorical variables are presented as n (%). HAMD-17: 17-item Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; mmHg: Millimeters of Mercury; CTQ: Childhood Trauma Questionnaire; SLE: Stressful life events questionnaire; PSQI: Pittsburgh Sleep Quality Index; BD I and II: Bipolar Disorder type I and II, respectively; N.A.: not available. *Illness duration was defined as time from first episode. **Untreated bipolar disorder was defined as time from first manic, hypomanic or mixed episode to time of diagnosis of bipolar disorder.

with healthy individuals. Further, in a post-hoc analysis, no difference was detected between the median [interquartile range] of hair cortisol concentrations in patients with BD of Asian ancestry and of Caucasian ancestry (11.9 [9.0–43.6] ng/mg hair vs. 8.8 [6.0–13.0] ng/mg hair, P = 0.09). To further validate our findings, we repeated our main model adjusted for sex and age, this time excluding the ten patients with BD of Asian or mixed Asian ancestry, and hair cortisol concentration remained elevated in patients with BD compared with healthy individuals (B = 1.312, 95%CI: 1.101–1.563, P = 0.002). Similarly, excluding the two outliers in addition to the ten patients with BD of Asian and mixed Asian ancestry, did not alter the findings (B = 1.252, 95%CI: 1.068–1.468, P = 0.006).

The results of the fully adjusted mixed effect regression analysis are shown in Table 2, lower part. When adjusting for sex, age, waist circumference, systolic blood pressure, childhood trauma, stressful life events, YMRS score, HAMD-17 score and sleep disturbances patients with BD had 43.5% higher hair cortisol concentrations compared with healthy individuals (B = 1.435, 95%CI: 1.078–1.911, P = 0.014). In this model unaffected relatives and healthy individuals had similar hair cortisol concentrations. In the model, age was associated with hair cortisol concentrations (B = 1.010, 95%CI: 1.001–1.020, P = 0.037) but there were no associations with other covariates.

3.3. Association between medication, illness and stress variables, respectively, and hair cortisol concentrations in patients with newly diagnosed/first episode bipolar disorder

In a multiple regression model, adjusted for age, sex, BD type, illness duration, waist circumference, systolic blood pressure, childhood trauma, stressful life events, YMRS score, HAMD-17 score, sleep

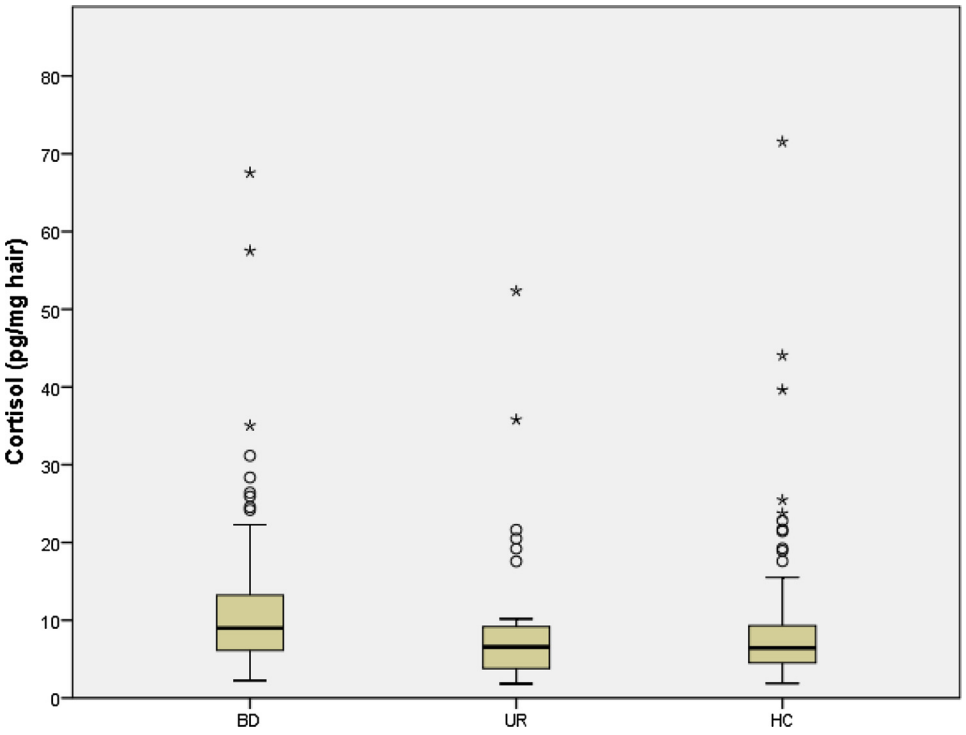

Fig. 1. Box plot of hair cortisol concentration in patients with newly diagnosed bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy individuals (HC). The lower and upper hinges correspond to the first and third quartiles and the upper and lower whiskers extend from the hinge to the largest and lower value, respectively, no further than 1.5 times the interquartile range from the hinge. Data beyond the end of the whiskers are plotted individually. For illustrative purposes, four outliers were not depicted in the figure, namely three patients with hair cortisol values of 177.8, 266.6 and 923.6 ng/mg hair, respectively, and one healthy individual with a hair cortisol concentration of 177.8 ng/mg hair.

Table 2
Hair cortisol concentrations in patients with bipolar disorder (BD) and their unaffected first-degree relatives (UR) compared with healthy individuals (HC).

Model		B	95%CI	P-value
1				
	Male vs. female sex	1.215	1.027-1.439	0.023
	Age	1.014	1.005-1.023	0.002
	BD vs. HC	1.351	1.130-1.615	0.001
	UR vs. HC	0.970	0.742-1.268	0.8
2				
a	Male vs. female sex	1.179	0.976-1.424	0.08
	Age	1.010	1.001-1.020	0.037
	BD vs. HC	1.435	1.078-1.911	0.014
	UR vs. HC	0.971	0.733-1.286	0.8
	Waist circumference	1.001	0.993-1.009	0.8
	Systolic blood pressure	1.005	0.998-1.011	0.2
	Childhood trauma (CTQ total score)	1.000	0.991-1.009	0.9
	Stressful life events (SLE 12 month score)	1.005	0.991-1.020	0.5
	Hamilton score (HAMD-17 total score)	0.992	0.975-1.009	0.4
	Young mania score (YMRS total score)	0.997	0.973-1.021	0.8
	Sleep disturbances (PSQI total score)	1.006	0.982-1.030	0.6

Model 1: Linear mixed effect regression analysis adjusted for group, age and sex with familial relationship as random effect. Model 2: Linear mixed effect regression analysis adjusted for group, age, sex, waist circumference, systolic blood pressure, childhood trauma, Stressful Life Events, scores on the HAMD-17 items and YMRS scales and sleep disturbances with familial relationship as random effect. B represents backtransformed beta values. CTQ: Childhood Trauma Questionnaire; SLE: Stressful Life Events; HAMD-17: Hamilton Depression Rating Scale-17 items; YMRS: Young Mania Rating Scale; PSQI: Pittsburgh Sleep Quality Index.

disturbances, lithium, antiepileptics, antidepressants and antipsychotics, we solely found an association of male sex and higher hair cortisol concentrations ($B = 1.342$ CI: 1.025–1.758, $P = 0.032$). Excluding the outliers with hair cortisol values above 200ng/mg hair did not change the results.

In a similar model, including all patients, we substituted the four treatment variables with the dichotomous variable current medication (yes/no). In this model, hair cortisol concentrations did not differ between patients with BD receiving medication and medication-free patients with BD.

Finally, we found no main effects of childhood trauma or stressful life events the preceding 12 months or severity of manic and depressive symptoms (YMRS score and HAMD-17 score) on hair cortisol concentrations in univariate analyses ($P > 0.05$).

4. Discussion

4.1. Main results

We found a higher hair cortisol concentration in 181 patients with newly diagnosed/first episode compared with 101 healthy individuals. This elevated concentration was neither explained by waist circumference nor systolic blood pressure, which in a recent meta-analysis were associated with increased hair cortisol concentrations (Stalder et al., 2017), or by other potential factors associated with disturbed HPA axis such as sleep disturbances (Manenschijn et al., 2011), childhood trauma (Belvederi Murri et al., 2016; Heim et al., 2008) or recent stressful life events (Staufenbiel et al., 2014) as adjustment for these variables did not alter the finding. In analysis among patients with BD, we did not find an association of hair cortisol concentrations and investigated stress measures or illness- and medication variables. Finally, hair cortisol concentrations did not differ among the 42 unaffected first-degree relatives and the 101 healthy individuals.

4.2. Comparison to other case-control studies

Our findings of elevated hair cortisol concentrations in patients newly diagnosed with BD are in line with results from a smaller study comparing patients with BD type I and healthy individuals (Streit et al., 2016), and contrast results from the other study where hair cortisol concentrations did not differ between patients with BD (type I and II) and healthy individuals (Manenschijn et al., 2012). However, the latter study found increased hair cortisol concentrations in older patients with BD with an onset > 30 years with median age [interquartile range] 50 [20–82] years compared with median age [interquartile range] in the present study 29 [24–36] years. It is possible, that cortisol

concentrations differ with stages and age periods of BD, as BD progress clinically with time (Kessing and Andersen, 2017).

4.3. Possible predictors of hair cortisol

4.3.1. Sex, age, waist circumference and systolic blood pressure

In our main analysis, increasing age and male sex were associated with higher hair cortisol concentrations in accordance with results from a recent meta-analysis of determinants of hair cortisol (Stalder et al., 2017) adding to validate our findings. However, in contrast to observations in the meta-analysis, we found no associations of hair cortisol concentrations and waist circumference or systolic blood pressure, respectively.

4.3.2. Stressful life events the preceding 12 months and childhood trauma

We included two measures of stress: stressful life events the preceding 12 months and childhood trauma. Recently, negative life events have been associated with increased hair cortisol concentrations in a prospective study of 71 patients with BD (Staufenbiel et al., 2014). Despite our findings of higher occurrence of stressful life events the preceding 12 months among patients with BD and their unaffected first-degree relatives compared with healthy individuals, we found no association between stressful life events and hair cortisol concentrations. Notably, the period reflected in the stressful life event questionnaire was 12 months, whereas hair cortisol concentrations reflected only 3 months, possibly contributing to the lack of association. However, our results comport with the result from the meta-analysis by Stalder et al. where no consistent associations with mood disorders and self-reports of perceived stress, depressiveness or social support were found (Stalder et al., 2017). Further, Stalder et al. (2017) found increased hair cortisol concentrations associated with ongoing chronic stress, but not with past stress (Stalder et al., 2017). We did not distinguish between ongoing and past stress; thus, we may possibly have overlooked such an association.

Similarly, against expectations based on previous findings within the HPA-axis (Belvederi Murri et al., 2016), we did not find an association of childhood trauma and hair cortisol concentrations, neither in analyses comparing groups nor in analyses within patients with BD. However, the influence of childhood trauma on the HPA-axis seem rather complex with a possible epigenetic component (Aas et al., 2016).

Our results indicate that even though childhood trauma and stressful life events the preceding 12 months were much more prevalent in patients with BD and in their unaffected first-degree relatives compared with healthy individuals, these stress measures were not associated with the elevated systemic hair cortisol concentrations we found in patients with BD. Several measures of stress are available and a recent review highlights that interview based assessments seem superior in stress evaluation (Harkness and Monroe, 2016).

4.3.3. Sleep disturbance

Contrasting the findings of increased hair cortisol concentrations in shift workers with disturbed circadian rhythms (Manenschijn et al., 2011) we did not observe an association between sleep disturbances and hair cortisol concentrations neither in analyses between groups nor in analyses within patients with BD. We assessed sleep disturbance during the past month using the standardized questionnaire PSQI (Buysse et al., 1989), whereas the hair cortisol concentration analysis did not include the past month, but more likely the preceding three months (LeBeau et al., 2011). It would have been more accurate to assess sleep disturbance during the same period as reflected in the hair sample, thus we cannot exclude a possible effect of disturbed circadian rhythms on our results.

4.3.4. Illness variables

We did not find any effect of illness related variables including BD subtype (BD type I vs. BD type II) or illness duration. Streit et al. (2016) investigated hair cortisol exclusively in patients with BD type I and found elevated concentrations, whereas Manenschijn et al. (2012) investigated patients with BD type I and type II and did not find increased hair cortisol concentrations (Manenschijn et al., 2012; Streit et al., 2016). Our findings suggest that at least in newly diagnosed BD concentrations of hair cortisol do not differ between BD type I and type II.

The association of illness duration and hair cortisol concentrations has not previously been investigated, and in our cohort of newly diagnosed patients with BD, we did not find an association. However, further research is required to determine whether illness duration and hair cortisol concentrations are associated. Similarly, we found no association between manic and depressive symptoms and hair cortisol concentrations in a fully adjusted regression model as well as in univariate analyses. Accordingly, in major depressive disorder, severity of depression was neither associated with hair cortisol concentrations in four prior studies as reviewed by Herane Vives et al. (2015). The only prior study in BD (Streit et al., 2016) investigating a possible association between hair cortisol and manic and depressive symptoms did not observe an association between depressive symptoms and hair cortisol concentrations. However, higher hair cortisol concentrations were associated with increasing manic symptoms in patients with BD (Streit et al., 2016). From this study, we cannot exclude an association, as ratings of mood symptoms were assessed based on the preceding three days and may vary from the mood symptoms experienced the three months reflected in the hair sample. Further, we may have overlooked a possible association between mood symptoms and hair cortisol concentrations since most patients with BD were in remission at the time of examination.

4.4. Hair cortisol in unaffected first-degree relatives of patients with bipolar disorder

Two recent large studies of hair cortisol and heritability found heritability of 50–72% (Rietschel et al., 2017; Tucker-Drob et al., 2017), however we observed similar hair cortisol concentrations in unaffected first-degree relatives of patients with BD and healthy individuals. Thus, our findings did not suggest that hair cortisol concentrations are associated with the familial risk for BD. However, our sample of at risk individuals was modest, potentially leading to type II errors.

The lack of difference in hair cortisol concentration between unaffected first-degree relatives and healthy individuals contrast prior observations of higher salivary cortisol concentrations in offspring of patients with BD than in offspring of individuals without mental illness (Ellenbogen et al., 2006, 2010). Our findings also conflict previous findings from our group of increased evening salivary cortisol concentrations in high-risk unaffected twins (co-twin with affective disorder) compared with twins not predisposed for affective disorder (Vinberg et al., 2008). This may be a result of the different measurement methods: hair cortisol being a measure of long-term cortisol secretion, whereas salivary cortisol is a measure of acutely circulating cortisol concentration (Stalder and Kirschbaum, 2012). Additionally, our first-degree relatives were older than the offspring in the studies by Ellenbogen et al. (Ellenbogen et al., 2011, 2006, 2010) and younger than participants in the study by Vinberg et al. (2008), thereby possibly further limiting the comparability between the studies.

Finally, we did not include relatives with major psychiatric illness and thus, our findings are restricted to unaffected relatives aged 15–40 years. Considering the age of the unaffected sample (median age [interquartile range]: 26 [22–31] years), some may have been beyond the typical age of onset of BD and in this way potentially not fully representing individuals at high risk which could ultimately reduce the chance of identifying risk markers for BD. Further studies are needed to clarify the role of the HPA-axis before onset of BD.

4.5. Strengths and limitations

We included a large group of well-characterized patients with newly diagnosed or first episode BD and their unaffected first-degree relatives. Our study population seemed representative of newly diagnosed patients with BD with a median age of illness onset of 17 years of age and a median delay in diagnosis of five years (Baldessarini et al., 2007; Kessler et al., 2007). Further, our observations clearly illustrate the challenge of recruiting patients early in their course of illness due to the well-known diagnostic delay of BD (Baldessarini et al., 2007).

There are some limitations to this study. Firstly, our sample of unaffected first-degree relatives was relatively small primarily caused by either first-degree relatives having a major psychiatric illness themselves or lacking consent from patients with BD to contact their relatives. Thus, we cannot exclude that the findings for unaffected first-degree relations reflect a type II error although B was close to one (as can be seen from Table 2: B = 0.970, 95%CI: 0.742–1.268). Notably, prior observed differences in salivary cortisol concentration between offspring of patients with BD compared with offspring of individuals without psychiatric illness were small, however, statistically significant higher in offspring of patients with BD (Ellenbogen et al., 2006, 2010). We aimed to detect possible alterations in first-degree relatives of the included patients with BD without mental illness at time of inclusion and to follow these unaffected relatives over time to detect possible biomarkers predicting onset of BD. Nonetheless, considering the age of the unaffected sample, some may have been beyond the typical age of onset of BD in this way potentially not fully representing individuals at high risk, which could ultimately reduce the chance of identifying risk markers for BD. Further, our exclusion criteria (e.g, a history of alcohol or substance abuse) may have been too restrictive and in this way limiting the sample size of first-degree relatives. In future studies of hair cortisol in first-degree relatives or offspring of patients with BD, it should be considered to include participants younger than the expected age of onset of BD in early adulthood (Kessler et al., 2007), hence presumably more accurately reflecting cortisol concentrations predating BD. Taken together, our observations in unaffected relatives should be interpreted with caution as we may possibly have overlooked a true difference.

Secondly, healthy blood donors can be regarded as a super healthy control group. The control group (i.e., healthy individuals) in this study had higher education level with less individual variation than did patients with BD. Nonetheless, considering the challenges of choosing a proper control group (e.g., avoidance of bias, representativeness of the general population) (Grimes and Schulz, 2005), the randomly recruited sex- and age-matched blood donors, from the same catchment area as patients with BD represent a pragmatic and valid control group. However, our findings should be replicated preferably with a population based control group before final conclusions are drawn.

Thirdly, we did not find an effect of current psychotropic medication, in contrast to a recent meta-analysis of the HPA-axis in BD, where antipsychotics were negatively associated with hair cortisol concentrations (Belvederi Murri et al., 2016). We cannot exclude a true effect of drug treatment as the number of patients within each of the four drug classes (lithium, antidepressants, antiepileptics, antipsychotics) were small. Further, when simplifying to the dichotomous variable psychotropic medication (yes/no) treatment effect may possibly have been belied if the individual treatment effects are not parallel.

Finally, while we adjusted our analyses for a number of potential confounders, we cannot exclude that residual confounding influenced our findings. For example, black hair color seems to be associated with higher levels of hair cortisol (Binz et al., 2018) and it is a limitation that we did not record hair color. Excluding the patients with BD of Asian or mixed Asian ancestry did not change the findings, however, we may possibly have overlooked a variation according to hair color among Caucasian.

4.6. Implications

In conclusion, we found that patients with newly diagnosed/first episode BD but not their unaffected first-degree relatives had elevated hair cortisol concentrations compared with healthy individuals. Potential confounders that may be associated with disturbed HPA axis including waist circumference, systolic blood pressure, sleep disturbances, childhood trauma or recent stressful life events did not explain this elevated concentration in patients with BD. Further, there were no association with psychotropic medication or illness related variables among patients with BD.

Our findings indicate the presence of physiological stress in early stages of BD. In the ongoing longitudinal Bipolar Illness Onset Study (BIO) (Kessing et al., 2017) we repeatedly measure hair cortisol concentration, which will add to the understanding of the role of stress before onset of BD and in different states and stages of BD.

Declaration of interest

KC, KM and FN declare no conflicts of interest. MV discloses within the last three years consultancy fees from Lundbeck. LVK reports receiving honoraria from Sunovion within the preceding three years.

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