Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders

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ABSTRACT

Background: The neural diathesis-stress model is useful to understand schizophrenia (SZ) and bipolar (BD) disorders. Childhood maltreatment could affect the Hypothalamic–Pituitary–Adrenal (HPA)-axis and lead to chronic changes in stress-sensitivity, which can be measured with hair cortisol concentrations (HCC), representing long-term, cumulative cortisol levels. Here we investigated if childhood trauma experiences are associated with chronic changes in the HPA axis in severe mental disorders.

Methods: Participants with SZ or BD (N=63) and healthy controls (N=94) were included, and HCC was measured by ELISA. History of childhood maltreatment was assessed using the Childhood Trauma Questionnaire (CTQ). Global function and symptom levels were obtained

using the Global Assessment of Functioning (GAF) Scale and the Positive and Negative Syndrome Scale (PANSS). A neuropsychological test battery (MATRICS) was performed to assess cognitive functions.

Results: Our study shows for the first time that patients with a history of childhood maltreatment have higher HCC relative to both healthy controls and patients without a history of childhood maltreatment (P=0.01, η_{p^2} =0.046). In addition, patients experiencing a mood episode had higher HCC than patients in remission (P=0.03). Lastly, we are the first to show that patients with higher HCC had poorer cognitive performance, specifically working memory (P=0.01). All associations were irrespective of diagnostic group. A factor analysis confirmed a subgroup within the patients characterized by childhood maltreatment and elevated HCC.

Conclusions: Findings support the neural diathesis-stress model in SZ and BD pointing to long-term changes in HPA-axis following childhood maltreatment experiences.

INTRODUCTION

Previous studies of patients with schizophrenia (SZ) or bipolar disorders (BD) have demonstrated that stress is an important factor in the development and maintenance of these disorders (Pruessner *et al.,* 2017; Nemeroff, 2016, Aas *et al.,* 2016). However, the biological mechanism by which stress affects severe mental disorders remain unclear. Cortisol level is the most frequently reported hypothalamic-pituitary-adrenal (HPA) axis variable, as it can easily be measured in blood, urine, and saliva. These well-established methods reflect transient circulating cortisol

levels, which can be influenced by several factors such in addition to stress the diurnal rhythm, patterns of activity or behavior and other variables that (follow the day-night cycle). Repeated measures can capture stress reactivity, changes in cortisol release during various types of stress, or the awakening response, as a marker of the dynamic HPA response to a naturalistic stressor (Mondelli *et al.*, 2010). However, as the traditional methods of blood, saliva, and urine sampling lack stability, they are not suitable for evaluation of chronic stress (Stalder and Kirschbaum, 2012). Given these challenges in obtaining reliable and valid estimates of long-term cortisol levels, a new method has emerged using human hair (Stalder and Kirschbaum, 2012). As cortisol is incorporated into the hair shaft during hair growth, the examination of cortisol in a specific hair segment is believed to provide a retrospective index of cumulative cortisol secretion over the period during which the hair segment has grown. Given that the average hair grows 1 cm per month (Wennig, 2000), the examination of a 3-cm segment of hair allows the assessment of cumulative cortisol exposure over a period of three months, a major advantage when measuring chronic cortisol exposure. Indeed, a recent HCC meta-analysis concluded that stress-exposed groups on a whole exhibit 22% increased HCC. This long-term cortisol hypersecretion emerges particularly when stress is still ongoing at the time of study (Stalden *et al.,* 2017).

Stressors, which can be physiological or psychological in nature, are often defined as allostasis (Russell *et al.*, 2012). The body's initial physiological reactions to stress are intended to maintain homeostasis. However, a long-term increase in allostatic load is associated with a wide range of adverse effects on neuroendocrine, cardiovascular, metabolic, and immune systems as well as on the brain and cognitive functions (Edes and Crews, 2017, Pruessner *et al.*, 2017). The sensory processing of stressful stimuli results in the release of corticotrophin releasing hormone (CRH) from the periventricular nucleus of the hypothalamus. CRH then stimulates the anterior pituitary to secrete adrenocorticotropin releasing

hormone (ACTH), which serves to trigger the secretion of cortisol by the adrenal glands. While acute cortisol reactivity constitutes an essential part of the adaptive response to challenge (Sapolsky, 2000, Wolf, 2003), chronic elevated cortisol levels seem to have harmful effects on the brain and cognitive function (Pruessner *et al.*, 2017, Sapolsky, 2000, Wolf, 2003), plausible by interacting with brain structure and function in specific regions. In 1968 McEwen and colleagues found that the area of highest density of glucocorticoid receptor was in the hippocampus, and area important for memory and cognitive function (McEwen *et al.,* 1968). . Interestingly a crucial effect of the HPA axis activity on the brain is via the glucocorticoids, which regulate the neuronal survival and neurogenesis in the hippocampus (Pariante and Lightmann, 2008). Glucocorticoids, on the other hand, suppress Brain-derived neurotrophic factor (BDNF) in the hippocampus, a protein important for neuronal survival (Nuernberg *et al.,* 2016). It could be suggested from the neuronal diathesis-stress perspective, that excessive exposure to stress is harmful for the brain, and psychopathology is a potential consequence of this exposure in vulnerable individuals (Pruessner *et al.*, 2017, Walker and Diforio, 1997).

Childhood maltreatment, a severe stressor, is a well-known risk factor for developing a wide range of severe mental illnesses, including psychotic disorders (Aas *et al.*, 2016, Varese *et al.*, 2012). A large survey of more than 9000 individuals demonstrated that severe childhood adversity accounts for nearly 32% of psychiatric disorders (Green *et al.*, 2010), including up to 44% of early-onset disorders. In fact, long-term changes to the HPA axis have been proposed to be a neurobiological characteristic linking childhood maltreatment to psychopathology (Nemeroff, 2016). A few studies have shown that childhood maltreatment alters the long-term stress response, potentially through epigenetic modifications (Klengel *et al.*, 2014), which is supported by studies showing changes in cortisol release following childhood maltreatment

experiences (Nemeroff, 2016). However, most studies have used one or a limited number of cortisol tests that could be influenced by variations in acute stress and the diurnal rhythm.

Based on the diathesis-stress perspective, high levels of stress may be characteristic of being in a current mood and/or psychotic episode (Pruessner *et al.,* 2017. However, the association between remission and non-remission status and HCC has not yet been established and more studies are needed. A recent study reported higher HCC in patients with BD compared to healthy controls (Streit *et al.*, 2016), with no significant differences between SZ group and the control group. However, replications are lacking, and due to the effect of childhood maltreatment on stress regulation, this needs to be investigated by use of the more stable HCC method. Studies investigating HCC and cognitive function in SZ and BD are also lacking. The literature shows variation in cortisol levels in patients with a severe mental disorder, suggesting heterogeneity in cortisol levels within patients' sample. No one has to our knowledge investigated if previous findings can be explained by biomarker subgroups within patients with a severe mental disorder, using HCC as a marker of HPA activity. Therefore, we decided to investigate if we could extract a subgroup of patients characterized by childhood maltreatment and elevated HCC. In the factor analyses we included clinical variables and cognitive variables associated with 1) having a diagnosis of schizophrenia or bipolar disorder (Aas *et al.,* 2014, Key *et al.,* 1987), 2) a history of childhood trauma (Aas *et al.,* 2016, Pruessner *et al.,* 2017), and 3) sensitive to HPA axis activity (Pruessner *et al.,* 2017).
Variation of cortisol levels within severe mental disorders may conflate subgroups of patients with different biomarker entities (Pruessner *et al.,* 2017), which we aim to clarify in this study. Biological heterogeneity within the patient population is supported by studies of both blunted and heightened cortisol in severe mental disorder, as well as abundant null findings (Pruessner *et al.,* 2017). Based on the stress-diathesis model we

hypothesized that patients with a severe mental disorder who report a history of childhood maltreatment represent a subgroup characterized by elevated HCC. Despite the plausibility of this hypothesis, no prior study has investigated this in a sample of SZ and BD. Secondly, because high levels of stress over time have been linked to cognitive and brain alterations (Wolf, 2003), we hypothesized that higher HCC would be associated with poorer working memory, long-term and short-term memory, and mental processing speed in severe mental disorders.

METHODS

Participants

The participants were recruited consecutively from psychiatric units (outpatient and inpatient) in four major hospitals in Oslo, as part of the Thematically Organized Psychosis (TOP) Study. Patients and controls were recruited from same catchment areas, with similar ethnical background (95% Caucasians). A total of 157 participants (with schizophrenia spectrum [n=28] or bipolar disorder spectrum [n=35] and healthy individuals [n=94]) were recruited. Inclusion criteria for the clinical groups included age range between 18 and 65 years, and a Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnosis of schizophrenia spectrum (SZ) or bipolar spectrum diagnosis (BD). Inclusion criteria for the healthy controls were the following: living in the same district as the patients, being between 18 and 65 years and having no lifetime diagnosis of any Axis I diagnosis, as assessed using the Structured Clinical Interview for DSM-IV (SCID, Spitzer et al.1992). Exclusion criteria for all groups included the following: organic psychosis, neurological disorder, and unstable or uncontrolled medical conditions interfering with brain function, and age outside the range of 18–65 years. Moderate to severe brain damage or IQ under 70 was also exclusion

criteria for all participants. The Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate approved the study. All the participants gave written informed consent. The participants were enrolled between April 2016 and December 2017.

Clinical Assessment

Psychiatrists, psychiatrists-in-training, medical trained professionals (MD), and clinical psychologists performed clinical assessments. Diagnoses were based on the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I), chapters A-E. All clinical personnel completed training in diagnostics and symptom rating based on the training program at The University of California, Los Angeles, UCLA. The diagnostic reliability of this ongoing study was found to be satisfactory with an overall agreement on DSM-IV diagnostic categories of 82% and an overall κ of 0.77 (95% CI: 0.60-0.94). Remission of psychotic symptoms was defined as Positive and Negative Syndrome Scale (PANSS) items scores below four of the following items: PANSS Positive (P) symptoms P1, P2, P3, PANSS general psychopathology (G) G9, G5, PANSS negative symptoms (N) N1, N4, and N6. Remission of affective episodes was based on the DSM criteria's. Symptom severity and function were rated separately using a split version of the Global Assessment of Functioning Scale (GAF; Pedersen *et al.,* 2007) dividing into GAF functioning and GAF symptoms. Duration of treatment was estimated based on current age and age at first treatment.

Childhood trauma

To assess the occurrence of childhood adverse events, we used the Childhood Trauma Questionnaire (CTQ), a retrospective questionnaire that assesses traumatic experiences in childhood. This self-report questionnaire with 28 items (Bernstein *et al.*, 1994) yields scores on five subscales of trauma on a Likert scale format, ranging from 1 to 5, ranging from never true, to very often true. Each subdomain includes 5 items and has a minimum score of 5 and a maximum score of 25, with a total CTQ score ranging from a minimum score of 25 to a maximum score of 125. The reliability and validity of the CTQ have been demonstrated previously (Aas *et al.*, 2014, Bernstein *et al.*, 1994). CTQ includes both abuse (emotional, sexual or physical), and neglect (comprised of both physical and emotional neglect). The consequences of inadequate input (eg, neglect/deprivation) and harmful input (eg, abuse/trauma) may have distinct influence on brain and biological development. The CTQ also includes MD scale to detect underreporting of childhood trauma on the CTQ. Three reverse statements are rated on a Likert scale, with high minimization present if the participant would not change anything about their family, their family was the best in the world and they had the 'perfect childhood.' Selecting 'very likely' for any of these statements award one point, allowing a score of 0–3. Bernstein and Fink (1998) stated that any score above 0 indicated minimization. Any scores from 1 to 3 on the CTQ's MD Scale suggests the possible underreporting of maltreatment (false negatives) (Bernstein and Fink, 1998). We have previously showed moderate to good internal consistency of the MD items as shown by a Cronbach's alpha coefficient of 0.75 (Church *et al.,* 2017). The validity of the MD scale has been estimated based on a high correlation with The BIDRs (Bernstein and Fink, 1998)».

Owing to the high correlation between retrospective information of childhood maltreatment using CTQ neglect and CTQ minimization/denial (MD) items (Church *et al.*, 2017, MacDonald *et al.*, 2015, MacDonald *et al.*, 2016), as well as potential different effects of abuse and neglect on brain functioning (Nemeroff, 2016) the main analyses (parametric and regression analyses), will be performed for abuse only. Table 1 (demographic overview) also includes the CTQ total score. For the main analyses, abuse variables (emotional abuse, sexual abuse and physical abuse) was analysed in two ways: firstly, abuse reaching predefined moderate to severe levels by Bernstein (1994) were collapsed into a dichotomous total childhood abuse score (yes, no trauma), defined by at least one subdomain of abuse reaching moderate to severe level (see supplementary Material S1). Secondly, data on abuse was presented as a continuous variable (emotional abuse, sexual abuse and physical abuse added together as one variable) with a minimum score of 15 and maximum score 75. The two approaches were employed to facilitate that a certain severity threshold of abuse was met to influence clinical and biological entities (Nemeroff, 2016). Secondly, to avoid losing the variance in the data, follow-up confirmatory analysis was conducted analysing childhood abuse as a continuous variable.
Information on clinical symptoms and functioning and information on childhood maltreatment was available in patients only.

Hair cortisol concentrations (HCC)

Hair was collected from the posterior vertex region on the head. The hair samples were wrapped in aluminium foil for protection and storage. Three-cm segment from the scalp end of each hair sample was cut (using surgical scissors) and 25mg of the hair samples was placed in a 4.5ml polypropylene copolymer (PPCO) vial. It was not always possible to obtain 25mg of hair from each participant's sample and in these

cases, we used the amount of hair available and the weight of each individual sample of hair was accounted for in the subsequent calculation of HCC. If any samples were shorter than 3cm then the whole length was used and the approximate length of the sample reported. From the samples 10% were extracted in duplicate, for each duplicate sample, two separate 25mg segments were taken from the scalp end and then each segment processed separately. The hair samples were then washed using isopropanol to remove external contaminants. 2ml of isopropanol was added to the sample, shaken for 3 minutes then removed; this step was then repeated with another 2ml isopropanol and then the hair was allowed to dry in a clean air environment for 48 hours. Once fully dry, five ceramic balls were added to each tube and the hair samples ground to a powder using Fast Prep-24 (MP Biomedicals, LLC). To extract cortisol, we added 2ml of methanol to each sample and incubated the samples for 24 hours rotating the samples constantly.

The hair, methanol and ceramic balls were decanted into a polypropylene tube (Sarstedt AG & Co, Germany) that separated the ceramic balls from the rest of the mixture. The tube was centrifuged at 1500 RCF to separate the ground hair and methanol and then again at 3000RCF to compact the hair powder to the bottom of the tube. 1.4ml of the clear methanol supernatant was then decanted into a 2ml polypropylene cryovial. Next, the methanol was removed using a vacuum centrifuge (Scan Speed 40, Labgene) and the tubes frozen at -80°C until required for the cortisol ELISA.

Cortisol levels were determined using a commercially available competitive ELISA (Salimetrics, US). Samples were thawed and reconstituted with 0.125ml of Salimetrics cortisol assay diluent and the samples were then assayed in accordance with the manufacturer's

protocol. The results were expressed as picograms of cortisol per milligram of hair. All participants provided usable hair samples and duplicate samples were analysed and these yield consistent results.

Neurocognitive test battery

Neurocognitive tests were collected from the MATRICS Consensus Cognitive Battery (Kern *et al.*, 2008, Nuechterlein *et al.*, 2008). Matrics and t-scores were used from the following tests: *Working memory:* Letter-number Span and Spatial Spann. *Processing speed:* Trail Making Test Part A, Word Fluency and the Brief Assessment of Cognition in Schizophrenia (BACS) Symbol Coding, and *Short-term and Long-term verbal memory* were measured using the Hopkins Verbal Learning Test (HVLT-R) at immediate and delayed recall. A short version of the *Wechsler Abbreviated Scale of Intelligence (WASI)* was used to assess general IQ. The short version was based on the Vocabulary and Matrix reasoning from the larger WASI battery (Wechsler, 2007).

Statistics

Statistical analyses were performed using the IBM SPSS software, Version 25 (IBM, 2017). Continuous variables are presented as mean ± standard deviation. Chi-square tests are used to compare categorical variables between patients and controls. Levene's tests were initially run to investigate if patients had larger HCC variance than the controls, as variation of cortisol levels within patients with a severe mental disorder may conflate subgroups of patients with different HPA axis entities (Pruessner *et al.,* 2017). As the CTQ data were not normally distributed

Mann-Whitney U tests were initially run to compare reports across groups (BD and SZ). CTQ and HCC were log transformed before included in the parametric and regression analyses.

Linear test of covariance (ANCOVA) was performed to analyse differences in HCC depending on childhood abuse experiences, adjusted for age and sex. For the ANCOVA analyses, information on childhood abuse from the CTQ was dichotomized into <or ≥ the moderate to severe cut-off score by Bernstein (1994), see Supplementary Material Table S1. Sixteen of the patients had a score of ≥ moderate to severe childhood maltreatment on at least one type of abuse, dividing the patients into a trauma and no trauma subgroup.

Multiple linear regressions were used to investigate the relationship between HCC and current clinical (GAF and PANSS) and cognitive features in SZ and BD and CTQ, childhood abuse measured as a continuous variable (ranging from 5 to 75). For all these analyses, data were adjusted for age, sex, and diagnostic group (SZ, BD). Assumptions for the regression analyses were found satisfying. A factor analysis, with varimax rotation was run to verify that HCC loaded on the same construct as the clinical (GAF, PANSS) and cognitive (working memory, processing speed, memory) domains and childhood maltreatment (measured as a continuous variable). Factors were included if Eigenvalues were higher than 1. Three factors had higher Eigenvalues than 1. Coefficients lower than 0.3 were suppressed (Child, 2016). Factors with Eigenvalues higher than 1was selected for inclusion in the cluster analysis. The threshold for statistical significance was set at P <0.05 with posthoc Bonferroni corrections.

RESULTS

Demographics of the sample

The mean age of the sample was 34 years. There were no statistically significant differences in age or sex between the groups (HC, BD, SZ). Participants were recruited from similar catchment area and there were no difference in education levels between groups, $F(2,156)=2.47$, $P=0.1$. Eighteen (12%) of the patients were currently in an episode. Patients with a SZ diagnosis had lower current IQ than BD ($P=0.04$) and HC ($P<0.001$). Patients with a SZ diagnosis had also higher PANSS scores, and lower GAF than the BD group, and were more likely to be in a current psychotic episode. There were no overall differences in being a in a mood episode or reports of childhood maltreatment in the SZ and BD group ($P>0.1$), but patients with BD were more likely to report a history of childhood emotional abuse compared to patients with a SZ diagnosis (Mann-Whitney U test, $U=233.5$, $P=0.03$).

-Please insert Table 1 around here-

Hair cortisol and childhood maltreatment

Levene's test confirmed larger variance of HCC in the patients (HCC, mean±SD=19.79±41.17) compared to the controls (HCC, 11.77±10.50), $F(1,155)=10.6$, $P=0.001$). BD had intermediate HCC levels (18.59±36.57), and SZ had the highest (21.31±46.96), however differences between groups did not reach statistical significant levels ($p<0.05$). Relative to healthy controls and to patients without childhood

maltreatment, patients with childhood maltreatment had elevated HCC, $F(1, 146)=6.98$, $P=0.01$, $\eta p^2 =0.046$, Cohen's $d=0.53$ and 0.47, respectively, see Figure 1). Analyses were adjusted for age, sex, and diagnosis (SZ, BD).

Findings of elevated HCC in patients with childhood maltreatment experiences (emotional, sexual and physical abuse) was supported by a linear relationship of higher score on childhood abuse and higher HCC, ß=0.36, $t(52)=2.38$, $P=0.02$, see Figure 2.

-Please insert Figure 1, 2 around here-

Hair cortisol concentration and clinical characteristics of SZ and BD

Patients in a current mood episode had higher HCC levels compared to euthymic patients, $F(1,62)=5.11$, $P=0.03$. Patients with lower GAF-S scores (indicative of more severe current symptoms) had a trend for higher HCC levels, β=-0.29, $t(56)=-2.02$, $P=0.05$. PANSS positive symptoms during the last seven days were associated with higher HCC, β=0.30, $t(56)=1.98$ $P=0.05$, see Table 2. All data above were adjusted for age, sex and diagnosis (SZ or BD). Dividing into diagnostic groups, findings were similar for both SZ and BD, however, the association was no longer statistical significant for either group (data not shown). No significant association was observed between PANSS negative symptoms

and HCC (p>0.1). Follow-up analyses within the patient sample revealed trend level association between HCC and childhood maltreatment after adjusting for GAF-S, ß=0.27, $t(56)=1.78$, $P=0.08$.

-Please insert Table 2 around here-

Hair cortisol concentration and cognitive function

Patients performed worse on cognitive domains compared to controls on all tests apart from immediate recall from the HVLT (see Supplemental Material, Table S2). Within the patient group, higher HCC was significantly associated with lower scores on working memory, ß =-0.33, $t(59)=-2.68$, $P=0.01$, see Table 3. All analyses were adjusted for age, sex and diagnosis (SZ, BD). All findings above were irrespective of diagnostic group. In controls, higher HCC was associated with a trend level of better performance on processing speed, see Supplementary Material Table S3.

-Please insert Table 3 around here-

Hair cortisol concentration, factor and cluster analyses

The factor analysis confirmed three different groups within the patient sample. One of these groups included HCC, childhood maltreatment and clinical features (symptoms and function) from the GAF (see Table 4). A negative correlation between working memory and HCC was confirmed (r(df)=0.28, P=0.04), however, as the correlation coefficient was lower than the 0.3 cutoff, working memory was removed from the final factor loading (Child, 2006). Thus, our findings point to a subgroup of patients within the larger patient group characterized by elevated HCC, childhood trauma experiences and poorer clinical functioning (measured by the GAF). In addition, the factor analysis included two factors within the patient population where clinical features were not correlated to childhood maltreatment or HCC.

Twenty-nine % of the patients were classified as part of cluster three. Patients in cluster three was characterised by clinical features of factor three. Cluster two (34% of the patients) were characterised by the clinical characteristics of factor two. Patients in Cluster 1 (34% of the patients) were characterised by clinical characteristics of factor one and factor three.

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DISCUSSION

Our study is the first to show that adult patients with a severe mental illness (SZ or BD) who report a history of childhood maltreatment have elevated cortisol measurements in the hair, suggesting chronic HPA axis abnormalities. The stress-diathesis model in severe mental disorders is well established (Pruessner *et al.*, 2017, Walker *et al.*, 1997), but studies have focused on cortisol measurements taken from saliva or blood, which can be affected by variations in acute stress and the diurnal rhythm (patterns of activity or behavior that follow the day-night cycle). Thus, our study contributes new knowledge to the field of long-term changes in the HPA axis following childhood maltreatment in severe mental disorders. As information on childhood trauma in controls were not available in the cohort, it is not possible to determine whether controls were exposed to maltreatment, and, if so, whether similar patterns exist among controls.

The present findings of an abnormal HPA axis in these patients were supported by elevated HCC in patients currently experiencing a mood episode and in patients with diminished cognitive functions (specifically working memory), and increased positive symptoms as assessed by the PANSS positive subscale (including symptoms of hallucinations and delusions). These findings were potentiated in those with low functioning, and when controlling for GAF, the relationship between HCC and childhood maltreatment became trend levels. It could be suggested that a history of childhood trauma is associated with long-term stress sensitization and poorer function after illness onset (Pruessner *et al.,* 2017, Aas *et al.,* 2016). This is supported by our recent study of patients with first-episode psychosis who reported a history of childhood trauma experiences had more severe symptoms at baseline and after 12 months (Aas *et al.,* 2016), compared to patients who did not report childhood trauma. Thus, our current cortisol findings may represent trait rather than state phenomena, with a pathway from childhood trauma experiences to HPA axis abnormalities and more severe symptomatology and poorer functioning in psychosis. All though previous studies have shown that both high

cortisol levels measuring in salivary or plasma during the day and blunted awakening response is associated with poorer cognitive functioning in psychosis (Pruessner *et al.,* 2017) our study is the first to show that higher cortisol levels measured in hair is associated with poorer working memory in schizophrenia and bipolar disorder. It is unlikely that our finding of poorer working memory in patients with higher HCC is due to chance alone as the finding is statistically significant also after adjusting for number of cognitive domains tests. We observed larger variation of HCC in the patients compared to controls, with no statistically significant different HCC mean levels after normalizing of the data. This is contrary to the study by Streit *et al.,* (2016) who showed elevated HCC in BD, but like their finding of no significant differences between SZ group and the control group. The difference in BD could be related to power, as more patients were included in the study by Strait and colleagues (N=220) compared to patients included in our study (N=63). Furthermore, our findings contrast with the study by Andrade and colleagues (2016) who reported higher HCC in first-episode psychosis. However, compared to the study by Andrade and colleagues our patients had longer duration of illness (median, five years of treatment), and medication can have a normalizing effect of HPA axis (Pruessner *et al.,* 2017).

Our study confirms a link between childhood maltreatment and cortisol levels in adulthood using the more stable marker of cortisol that has accumulated over several months in the hairs. Our data highlight the importance of further research into HPA axis and clinical and cognitive impairments in SZ and BD. In addition to experiencing more frequently childhood traumatic events than healthy individuals (Varese *et al.,* 2012, Church *et al.,* 2017), patients with SZ and BD may also process stress differently owing to lower cognitive load and reduction in grey matter in regions critically implicated in acute stress responses and stress regulation (Allen *al et al.,* 2016, Anacker and Hen *et al.,* 2017, Wolf *et al.,* 2003, Rimol *et al.*, 2010, Velakoulis *et al.*, 2006), which may interact with a chronic or acute stress influence of these areas (Wolf, 2003,

Sapolsky, 2000). It could also be suggested that patients with psychosis are more vulnerable to chronic stress to due lower BDNF levels. It is possible that reduced BDNF plasma levels prior to childhood trauma worsen the adverse effect of childhood trauma experiences on brain development, accompanied by more severe neurodevelopmental changes following trauma experiences (Aas *et al.,* 2018). Future studies are needed to test this hypothesis.

Larger variation of HCC was observed within the patient group compared to healthy controls, indicating biomarker subgroups within the patients' sample. The larger variation in HCC in the patients than the controls could camouflage abnormalities in the HPA axis, namely both that hypo and hyper functioning is present within the patients' sample. Our study identified a subgroup of patients with a severe mental disorder (SZ and BD) characterized by a history of childhood maltreatment experiences and changes in the endocrine system (elevated cortisol levels measured in hair, HCC). Thus, our findings point to a subgroup of patients within the larger patient group characterized by elevated HCC, childhood trauma experiences and poorer clinical functioning (measured by the GAF). In addition, the factor and cluster analyses included patients with clinical features that were not correlated with HCC or childhood maltreatment experiences; hence our data suggest mechanistic or biological heterogeneity within the patient population. Our findings may be of value when interpreting conflicting cortisol findings from different studies, as studies may compare patients that are part of different biomarker entities.

Highlighting the importance of stress in disease course, stress management programs for psychosis have shown a reduction in hospital admission in the year following the intervention (Norman *et al.*, 2002). Thus, our data support the recent review by Pruessner at al. (2017), regarding a critical need for intervention studies investigating the effectiveness of alternative pharmacological and psychosocial interventions to

alleviate subjective and physiological stress response, with the ultimate goal of reducing the risk for psychosis onset and progression. Knowledge about inter-individual differences in stress vulnerability and psychosis and mood episodes can lead to the development of targeted interventions and assessment of their effectiveness.

Limitations: Childhood maltreatment was assessed using the Childhood Trauma Questionnaire, which is a retrospective instrument. However, the retrospective collection of childhood maltreatment in this population has been found to be a valid approach to collect such data, correlating with case notes (Widom *et al.*, 2008, Widom *et al.*, 2005). As we did not have data on childhood maltreatment from the CTQ in the control sample, we were not able to investigate if childhood maltreatment had a different long-term association to HCC in patients than the controls, however the variance of HCC in the control sample was small (SD=10.5), compared to the patients (SD=51.6), indicating less variance of HCC in the control sample compared to the patients. It should also be mentioned that out sample was relatively modest (63 patients and 94 healthy controls). The small N may have influenced the factor analysis. Furthermore, we did not have detailed information on medication (apart from years of treatment) for this sample, thus we were not able to adjust for potential effect of medication on HPA axis (Pruessner et al., 2017). Lastly our findings should be interpreted with caution until replicated in independent samples.

Conclusion: Our study identified a subgroup of patients with a severe mental disorder characterized by elevated HCC, childhood maltreatment experiences and more severe current psychopathology. These findings support the concept of chronic HPA axis dysregulation in a subgroup of patients with severe mental disorders.

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CONFLICT OF INTEREST

Over the past 3 years, DAP has received consulting fees from Akili Interactive Labs, BlackThorn Therapeutics, Boehringer Ingelheim, Posit Science, and Takeda Pharmaceuticals USA for activities unrelated to the current study. OAA has received speaker's honorarium from Lundbeck. All other authors report no biomedical financial interests.

CONTRIBUTORS

Monica Aas, Diego A Pizzagalli and Ole A Andreassen wrote the first draft. All authors contributed and approved the final version of the Manuscript.

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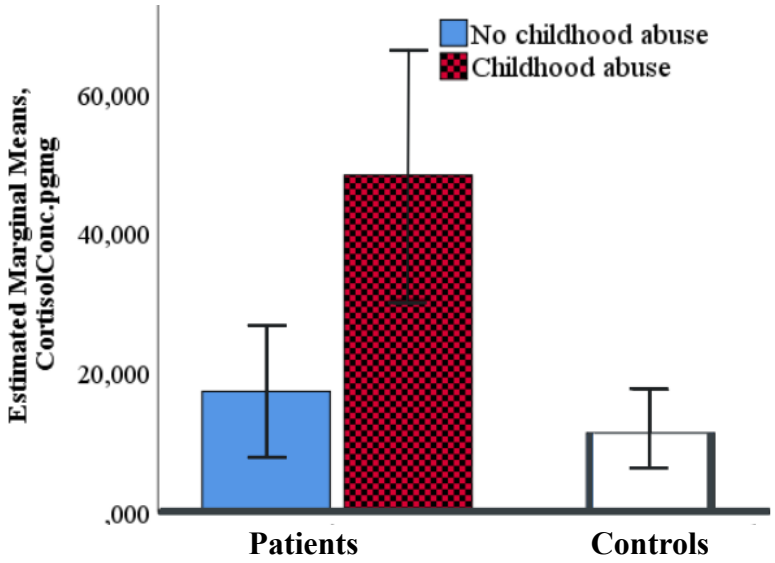
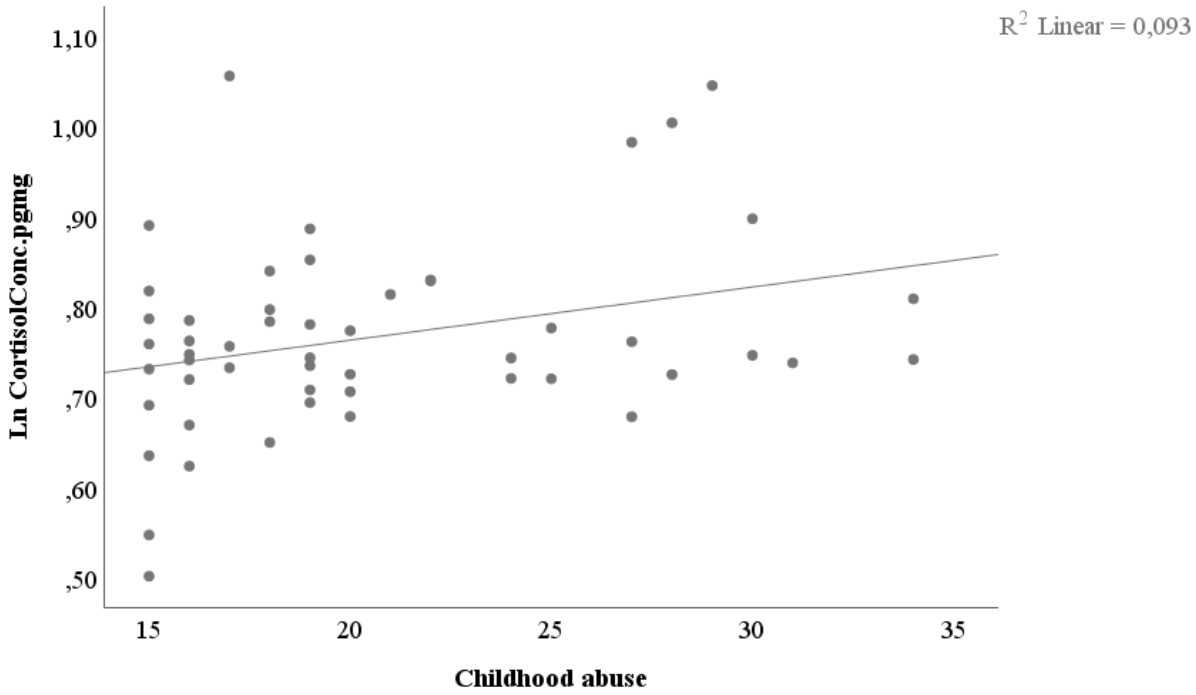
Figure 1: Patients with childhood maltreatment had higher hair cortisol concentration relative to patients without childhood maltreatment and to healthy controls



ANCOVA, $F(1,146)=6.98$, $P=0.01$. Data were adjusted for age and sex. Patients with childhood maltreatment compared to patients without maltreatment, Cohen's d=0.47; Patients with maltreatment compared to healthy controls, Cohen's d=0.53.

Figure 2: Patients with more severe childhood maltreatment from the CTQ had higher hair cortisol concentration



Linear regression, ß =0.36, t=2.38 p=0.02. Ln=log transformation. CTQ=Childhood Trauma Questionnaire. Childhood maltreatment = emotional abuse, sexual abuse and physical abuse from the CTQ.

Table 1. Demographics of the sample

	SZ N=28	BD N=35	HC N=94	Statistics
Age, mean±SD	33.6±11.6	32.1±10.8	35.3±9.5	*F*=1.20, df=2,156, *P*=0.3
Sex, N (%) Males	14 (53.8)	14 (40.0)	42 (51.2)	X^2=1.54, df=2, 156, *P*=0.5
Years of education in years, mean±SD	14.6±3.3	16.0±2.6	15.1±2.1	*F*=2.47, df=2,154, *P*=0.1
GAF-S, mean±SD	58.4±13.8	63.8±9.4	------	T=-1.85, df=1,59, *P*=0.07
GAF-F, mean±SD	53.5±16.2	63.2±11.4	------	T=-2.60, df=1,59, *P*=0.01
PANSS positive score, mean±SD	11.9±3.5	8.7±2.0	------	T=4.60, df=1,59, *P*<0.001
PANSS negative score, mean±SD	14.3±5.7	9.1±2.0	------	T=5.00, df=1,59, *P*<0.001
Childhood maltreatment*				
CTQ, Total score, median (min-max)	34 (25-49)	37 (25-49)	------	U=213.5, df=1, 55, *P*=0.10
Emotional abuse, median (min-max)	6 (5-15)	9 (5-24)	------	U=233.5, df=1, 55, *P*=0.03
Sexual abuse, median (min-max)	5 (5-9)	5 (5-13)	------	U=340.0, df=1, 55, *P*=0.83
Physical abuse, median (min-max)	5 (5-9)	5 (5-13)	------	U=343.0, df=1, 55, *P*=0.83

HC=healthy controls; SZ=schizophrenia; BD=bipolar disorder; HCC= hair cortisol concentrations; GAF-S= Global Assessment of Functioning scale, symptoms; GAF-F= Global Assessment of Functioning scale, functioning; PANSS=Positive and Negative Syndrome Scale; CTQ= Childhood Trauma Questionnaire. *= Mann-Whitney U Test. One hundred and fifty (96%) had data on years of education. Sixty (95%) of the patients had data on GAF and PANSS. Fifty six (89%) of the patients had data on childhood maltreatment.

Table 2. Patients with higher HCC had more positive symptoms (PANSS) and more general symptoms (GAF)

	β	t	df	*P* value
GAF F	-0.28	-1.82	56	0.08
GAF S	-0.29	-2.02	56	**0.05**
PANSS negative symptoms	0.01	0.08	56	0.94
PANSS positive symptoms	0.30	1.97	56	**0.05**

Analyses were corrected for age, diagnosis (SZ, BD) and sex.
HCC= hair cortisol concentrations; GAF-S= Global Assessment of Functioning scale and symptoms; GAF-F= Global Assessment of Functioning-Function; PANSS= Positive and Negative Syndrome Scale.

Table 3. HCC and cognitive functioning

	Patients			
	β	t	df	*P* value
Working memory	-0.33	- 2.68	59	**0.01**
Short-term memory	-0.16	-1.22	59	0.23
Long-term memory	-0.20	-1.47	59	0.15
Processing speed	-0.12	-0.96	59	0.34

Data were corrected for age, gender and diagnosis (SZ, BD).

Table 4. Factor loadings for rotated solution (three factors)

Rotated Component Matrix

	Component		
	1	2	**3**
Short-term memory	.92		
Long-term memory	.84		
Working memory	.81		
Processing speed	.62	-.34	
PANSS General psychopathology (G)		.87	
PANSS Negative symptoms (N)		.79	
PANSS Positive symptoms (P)		.62	
GAF symptoms		-.49	**-.75**
HCC			**.73**
Childhood abuse	.36		**.69**
GAF functioning		-.49	**-.66**

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
HCC= Hair cortisol concentrations, GAF= Global Assessment of Functioning; PANSS=Positive and Negative Syndrome scale.

Supplementary Material

Table S1. CTQ moderate to severe cutoff score for maltreatment

CTQ, Childhood maltreatment subtypes	Moderate to severe cutoff
Physical abuse	≥10
Sexual abuse	≥8
Emotional abuse	≥13

For estimates of frequencies of childhood maltreatment, we used the moderate to severe predefined cutoff suggested by Bernstein (Bernstein and Fink, 1998).

Table S2. Cognitive functioning and group status

	SZ mean±SE	BD mean±SE	Controls mean±SE	Statistics	Posthoc tests
Working memory	45.1±1.9	48.3±1.7	55.1±1.1	F=13.39, df=2, p<0.001	HC>SZ, BD
Short-term memory	49.3±10.0	51.5±8.9	51.3±9.8	F=0.55, df=2, p=0.58	ns
Long-term memory	44.9±11.5	49.6±9.2	51.7±8.8	F=4.2, df=2, p=0.02	HC>SZ
Processing speed	42.05±2.1	51.82±1.8	54.26±1.2	F=12.98, df=2, p<0.001	HC>SZ, BD>SZ

ANCOVA. Data were corrected for age, and sex. Mean and standard error (SE) is based on T scores. SZ=schizophrenia, BD= bipolar disorders, HC=healthy controls

Table S3. HCC and cognitive function in the control sample.

	Controls			
	β	t	df	*P* value
Working memory	0.15	1.30	91	0.20
Short-term memory	0.05	0.40	91	0.69
Long-term memory	0.13	1.12	91	0.27
Processing speed	0.22	2.01	91	**0.05**

Data were corrected for age, and gender.