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Mitochondrial DNA as a marker for treatment-response in post-traumatic stress disorder

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a serious mental health condition thought to be mediated by a dysregulated stress response system. Stress, especially chronic stress, affects mitochondrial activity and their efficiency in duplicating their genomes. Human cells contain numerous mitochondria that harbor multiple copies of their own genome, which consist of a mixture of wild type and variant mtDNA - a condition known as mitochondrial heteroplasmy. Number of mitochondrial genomes in a cell and the degree of heteroplasmy may serve as an indicator of mitochondrial allostatic load. Changes in mtDNA copy number and the proportion of variant mtDNA may be related to mental disorders and symptom severity, suggesting an involvement of mitochondrial dysfunction also in PTSD. Therefore, we examined number and composition of mitochondrial DNA before and after six weeks of inpatient psychotherapy treatment in a cohort of 60 female PTSD patients. We extracted DNA from isolated monocytes before and after inpatient treatment and quantified cellular mtDNA using multiplex qPCR. We hypothesized that treatment would lead to changes in cellular mtDNA levels and that change in mtDNA level would be associated with PTSD symptom severity and treatment response. It could be shown that mtDNA copy number and the ratio of variant mtDNA decreased during therapy, however, this change did not correlate with treatment response. Our results suggest that inpatient treatment can reduce signs of mitochondrial allostatic load, which could have beneficial effects on mental health. The quantification of mtDNA and the determination of cellular heteroplasmy could represent valuable biomarkers for the molecular characterization of mental disorders in the future.

1. Introduction

Post-traumatic stress disorder (PTSD) is a serious condition that can occur as a result of traumatic life events e.g., effective or potential life threat, serious injury and/or sexual violence; (APA, 2013). The symptoms are numerous and include intrusions, avoidance, and hyperarousal (diagnostic criteria for PTSD (F43.1), International Classification of Diseases, 10th revision, WHO, 1993) and are further characterized by intense, troubling thoughts and feelings related to the trauma experienced, which may persist long after the traumatic event has ended. Flashbacks, nightmares and reliving the traumatic situation as well as feelings of sadness and fear are typical. People with PTSD may avoid situations or people that remind them of the traumatic event. Approximately 3.5% of the adult American population suffers from PTSD annually, with women being affected two to three times more often as men (Lanius and Olff, 2017; Olff, 2017). Although the majority of individuals experience traumatic situation throughout their life span (around 50%–70%; Atwoli et al., 2015), not everyone develops PTSD. Furthermore, not every patient with PTSD responds to treatment and it is still unclear which biological factors are responsible for the risk of developing PTSD and the response to treatment. Many studies have observed dysregulations of the stress response system as well as the immune system in patients with PTSD (as reviewed by Kim et al., 2020; Mondelli and Vernon, 2019; Speer et al., 2019). In addition,

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genome-wide as well as epigenome-wide studies have already provided the first candidate genes and insights for the development of PTSD (Gelernter et al., 2019; Logue et al., 2020; Smith et al., 2020; Stein et al., 2021; Xie et al., 2013), but a more holistic approach may be necessary for a better understanding of the complex pathogenesis. One complementary approach could focus on mitochondria, which are increasingly being investigated in psychiatric research (Jou et al., 2009; Kasahara and Kato, 2018; Shao et al., 2008).

Mitochondria are double-membraned intracellular organelles which emerged from an endosymbiotic relationship between oxygen-consuming bacteria and eukaryotic cells (Wallace, 1992, 2005). They are the power stations of eukaryotic cells and generate energy via adenosine triphosphate (ATP) synthesis, which is mediated by the mechanism of oxidative phosphorylation (Castellani et al., 2020; Picard and McEwen, 2018a, 2018b; Picard et al., 2018). Mitochondria are also involved in bioenergetic processing as well as various homeostatic, apoptotic and signaling pathways (Wallace, 1992, 2005). A side product of oxidative phosphorylation is reactive oxygen species (ROS) which can increase under high cellular stress conditions. Augmented ROS production, amongst other markers, seems to be related to many diseases, pathological issues, and cell death (Kuznetsov et al., 2011). Moreover, mitochondria are very sensitive to environmental and metabolic stressors, such as glucocorticoids (Psarra and Sekeris, 2011), estrogen (Psarra et al., 2006a; b) and cannabinoids (Hebert-Chatelain et al., 2016). In the last decade, mitochondria and their contribution to sickness and health have been widely discussed (Picard et al., 2016). Especially their sensitivity to glucocorticoids and their involvement in glucocorticoid synthesis are interesting regarding stress exposure and the effects of stress on mitochondrial function and thus potential negative health outcomes (Picard et al., 2014). Mitochondrial dysfunctions have therefore sparked interest in research into a range of mental disorders such as PTSD.

Allostasis is the active process of an organism adapting to stress via different systems working together to maintain homeostasis. Mitochondria contribute to allostasis, but also conduct their own allostasis called mitochondrial allostatic load (MAL). Psychosocial stressors such as a low socioeconomic status, adverse childhood experiences and stressful life events or psychological problems contribute to MAL (Picard and McEwen, 2018a, 2018b). MAL can cause excessive ROS production and might provoke the release of chromatin remodeling signals that alter gene expression and can cause an energy production deficiency which increases cellular stress. This may lead to mitochondrial DNA (mtDNA) damage which can accumulate mtDNA mutations or mtDNA deletions, that altogether contribute to higher disease risk (reviewed by Daniels et al., 2020).

Mitochondria house their own circular, double-stranded genomes of 16.5 kb length, encoding for 37 genes. Each cell contains various copies of mitochondria, and each mitochondrion contains multiple DNA copies (mtDNA copy number; mtDNAcn). In addition, variant mtDNA copies are present within a cell, a condition termed heteroplasmy. A single cell can host a mixture of wild type and variant mtDNA including SNPs ($mtDNA_{mut}$) and deletions ($mtDNA_{del}$). As Filograna et al. (2021) described, wild type and mutated mitochondrial genomes coexist, and mutant mtDNA copies are tolerated as long as they do not exceed a certain threshold. If the threshold is surpassed, the mitochondrion is classified as dysfunctional with implications for its energy production capacities. A common deletion of 4977 bp has often been found in various tissues, associated with age and in high energetic tissues (Lee et al., 2010; Miller and Bennett, 2010; Phillips et al., 2014a; Reguly et al., 2010). As elaborated above, mtDNA mutations may accumulate under mitochondrial stress and may be indicators of MAL (Picard et al., 2014, 2018, 2019; Picard and McEwen, 2018a, 2018b). Mutations in the mitochondrial genome can lead to severe diseases which are often found associated with psychiatric comorbidities like depression or anxiety (reviewed by Anglin et al., 2012). Furthermore, associations of increased or decreased mtDNAcn in mental disorders have been examined by various studies. Altered $mtDNA_{cn}$ was found in patients with major depressive disorder (MDD; Cai et al., 2015; Chang et al., 2015), bipolar disorder (BD; Chung et al., 2022; Rollins et al., 2018), schizophrenia (SZ; Roberts, 2017; Rollins et al., 2018) and PTSD (Bersani et al., 2016). Moreover, there are findings that the response to psychological treatment or medication intake can lead to a change in $mtDNA_{cn}$ in patients with mental disorders (Nicod et al., 2016; Wang et al., 2017).

This study focused on PTSD, since one of the main characteristics of this mental disorder is an impaired stress response because of traumatic life events. Alteration of long-term hypothalamic–pituitary–adrenal (HPA) axis function in PTSD patients seems to be a hallmark of the disorder. Early studies by Yehuda et al. found lower levels of cortisol in PTSD patients suggesting hypocortisolism (Yehuda et al., 1990, 1996). Meanwhile, several other studies confirmed these findings (Neylan et al., 2005; Rohleder et al., 2004; Yehuda, 2005) while others failed to replicate these results (Bremner et al., 2007; Klaassens et al., 2012; Meewisse et al., 2007). Mitochondria are sensitive to stressors and play a crucial role in stress-signaling pathways such as the HPA axis and connections to mitochondrial involvement in PTSD and psychological trauma have already been described (Bersani et al., 2016; Daniels et al., 2020; Preston et al., 2018). According to Preston and colleagues (Preston et al., 2018), there are certain inherited and/or acquired mitochondrial risk factors, which disrupt mitochondrial function and therefore influence ATP production capacities, ROS production or dynamic changes in morphology and functionality. So far, only a few genetic studies investigating PTSD have been focusing on mtDNA. In a mitochondrial genome-wide association study, Flaquer et al. (2015) identified two mitochondrial SNPs (mt8414C→T and mt12501G→A) to be significantly associated with PTSD in a cohort of 1238 individuals. Bersani et al. (2016) examined a sample of male war veterans with PTSD and found $mtDNA_{cn}$ in granulocytes significantly lowered in PTSD patients compared to healthy controls. These findings suggest that $mtDNA_{cn}$ levels vary due to external stressors and changes in $mtDNA_{cn}$ might be related to changing symptom-severity, medication, and treatment-response.

Current research shows clear hints for mitochondrial dysfunction in PTSD symptomatology and etiology but further studies monitoring $mtDNA_{cn}$ during psychotherapy are required. However, a potential confounder already known from epigenetic research is cell-heterogeneity (Kumsta, 2019; Moser et al., 2020). To circumvent the problem of cellular heterogeneity this study was performed using purified monocytes to investigate $mtDNA_{cn}$ in a cohort of female PTSD patients before and after six weeks of inpatient treatment. Moreover, this study also considered levels of heteroplasmy during therapy focusing on a common deletion of 4977 bp in the mitochondrial genome ($mtDNA_{del}$). The research question was whether potential changes in $mtDNA_{cn}$ and/or $mtDNA_{del}$ can be observed over a period of six weeks, and if they can be related to symptom-severity and treatment-response. We hypothesized that $mtDNA_{cn}$ levels will change pre- to post-treatment and that these changes correlate with treatment-response. Secondly, we hypothesized that pre- and post-treatment levels of $mtDNA_{del}$ and the proportion of mitochondrial genomes with deletion in the total number of mitochondrial genomes ($mtDNA_{del}/mtDNA_{cn}$ ratio; mtDNA deletion ratio) will differ, which will also be correlated with treatment-response.

2. Methods

2.1. Sample characteristics

Sixty female patients of European descent with an age range from 20 to 60 years (M=40.03, SD=11.86) seeking PTSD inpatient treatment were recruited from the Department of Psychosomatic Medicine and Psychotherapy at the LWL-University Hospital, Ruhr-University Bochum. A current unremitted PTSD diagnosis and female sex were the inclusion criteria. Further sample characteristics including

comorbidities and medication are listed in the supplemental material (Suppl. Table SA1).

The study was approved by the Ethics Committee of the Faculty of Psychology at the Ruhr University Bochum (No. 155). The patients were informed in detail about the objectives of the study and their written consent was obtained.

2.2. Diagnosis and treatment

Prior to inpatient treatment admittance, diagnoses were made using the ICD-10 (F43.1; International Classification of Diseases, 10th revision, WHO, 1993) via a structured clinical interview taken in the outpatient department of the clinic. Moreover, PTSD symptoms were captured before and after inpatient treatment with the PTSD-Check List for DSM-5 (PCL-5; (Kruger-Gottschalk et al., 2017; Weathers et al., 2013). The PTSD patients received standard inpatient treatment. The mean treatment duration was 6.5 weeks (SD = 1.4) and involved cognitive behavioral therapy once every week, two sessions of trauma stabilization group therapy, three sessions of trauma group therapy and one session of a 'skills group'. Additionally, two sessions both of kinesitherapy, art therapy and physiotherapy, clinical rounds and daily short sessions with a clinical nurse were included. Individual therapy sessions consisted of different trauma exposure methods.

2.3. Laboratory procedure

Blood samples were collected by venipuncture in the morning before 9 a.m. (S-Monovette 9 ml K3E, Sarstedt). Monocytes were immunomagnetically purified from whole blood using the MACS System (Miltenyi Biotec, Bergisch Gladbach, Germany) before they were shock frozen and stored at − 80 °C. The homogeneity of the cells was determined with the BD FACSCanto TM II Flow Cytometer (BD Biosciences, San Jose, CA, USA), and showed high purity (98.3% ± 2.4% (SD). The DNA was isolated using the AllPrep RNA/DNA Mini Kit (Qiagen, Hilden, Germany). $MtDNA_{cn}$ and $mtDNA_{del}$ was quantified on a CFX384 Real-Time Cycler (BioRad, Hercules, USA), following a protocol as described by Phillips et al. (2014b). Further information on the PCR-protocol can be found in the supplemental material (Suppl. Table SA2).

2.4. Variables relevant for statistical analysis

PCL-5 total scores of each patient before (PCL-pre) and after (PCL-post) treatment represent PTSD symptom-severity, with higher scores indicating greater severity (0–80 points). Additionally, the response to therapy was measured by subtracting PCL-5 total scores from these two time points (Diff-PCL). $MtDNA_{cn}$ and $mtDNA_{del}$ were calculated as described by Phillips et al. (2014b) for both pre- and post-treatment. Furthermore, a difference in $mtDNA_{cn}$ and $mtDNA_{del}$ was calculated from pre- to post-treatment to indicate the amount of change in copy number (Diff-$mtDNA_{cn}$) and deletion (Diff-$mtDNA_{del}$) levels. The same procedure was applied for the mtDNA deletion ratio (Diff-$mtDNA_{del\ ratio}$), representing changes in heteroplasmy levels. Other variables relevant for statistical analyses are $mtDNA_{cn}$ at baseline (pre-$mtDNA_{cn}$) and post-treatment (post-$mtDNA_{cn}$), total number of mitochondria with the deletion at baseline (pre-$mtDNA_{del}$) and post-treatment (post--$mtDNA_{del}$), mtDNA deletion ratio at baseline (pre-$mtDNA_{del\ ratio}$) and post-treatment (post-$mtDNA_{del\ ratio}$) as well as age and BMI at baseline. In addition, changes in psychotropic medication were assessed during the PTSD inpatient treatment and included as a dichotomous variable (0 = no medication change; 1 = medication change).

2.5. Statistical analysis

Pre- to post-treatment differences in $mtDNA_{cn}$, $mtDNA_{del}$, mtDNA deletion ratio and PCL-5 total scores were analyzed using either a paired t-test or a Wilcoxon signed-rank test. Differences in Diff-$mtDNA_{cn}$ and Diff-$mtDNA_{del}$ levels between the two categories of psychotropic medication change were tested using Wilcoxon rank sum tests. Violations of the assumption of normality were assessed using Anderson-Darling's normality test. Effect sizes were determined using Cohen's *d* (Cohen, 1992). Spearman's rank coefficients of correlation were generated for all mitochondrial markers (pre-$mtDNA_{cn}$, post-$mtDNA_{cn}$, Diff-$mtDNA_{cn}$, pre-$mtDNA_{del}$, post-$mtDNA_{del}$, Diff-$mtDNA_{del}$, pre- $mtDNA_{del\ ratio}$, post $mtDNA_{del\ ratio}$, Diff- $mtDNA_{del\ ratio}$) and symptom-severity (PCL-pre, PCL-post, Diff-PCL). Correlations were adjusted for multiple comparisons using Holm's correction (Holm, 1979). Moreover, a multiple regression analysis was performed to determine whether changes in copy number levels and changes in deletion levels can predict changes in symptom-severity. Age, BMI, and changes in psychotropic medication were added as control variables in this model. Unstandardized regression coefficients represent changes in PCL-5 total scores from pre- to post-treatment while increasing pre-post differences in mtDNA copy number or mtDNA with deletion by one unit. All statistical analyses were performed in R (version 4.0.5) and R studio (version 1.4.1106) with a significance-level of $\alpha = 0.05$.

3. Results

Initially, data was available for 60 PTSD patients, but after removing all missing values, the sample was reduced to a size of n = 55. Table 1 presents descriptive data for all variables used in the statistical analysis.

3.1. Pre-post differences

The distributions of the copy number of mitochondrial genomes per monocyte ($mtDNA_{cn}$), copy number of mitochondrial genomes with deletion ($mtDNA_{del}$), and proportion of mitochondrial genomes with deletion in the total number of mitochondrial genomes ($mtDNA_{del\ ratio}$) differed significantly from a normal distribution and were therefore analyzed using Wilcoxon signed-rank tests. The Anderson-Darling normality test did not show any significant deviations for PCL-5 total scores which were hence tested with a paired t-test. Mean $mtDNA_{cn}$ decreased significantly from pre- to post-treatment as shown in Fig. 1A (*Ms* = 218.75 vs. 211.36, *SDs* = 44.83 vs. 54.47) $V = 941$, $p = .046$, $d = 0.15$, 95% CI [− 0.11, 0.40]. An effect size of $d = 0.15$ suggests a small effect. $MtDNA_{del}$ showed a significant decrease from pre- to post-treatment as well (Fig. 1B; *Ms* = 115.11 vs. 104.73, *SDs* = 44.09 vs. 45.52), $V = 1035.5$, $p = .03$, $d = 0.23$, 95% CI [− 0.03, 0.50]. Pre-$mtDNA_{del\ ratio}$ was significantly higher than post- $mtDNA_{del\ ratio}$ (*Ms* =

Table 1
Descriptive data for $N = 55$ participants.

Variable	Mean	SD	Median	Min	Max
Pre-$mtDNA_{cn}$	218.75	44.83	222	142	295
Post-$mtDNA_{cn}$	211.36	54.47	212	95	335
Pre-$mtDNA_{del}$	115.11	44.09	124	30	192
Post-$mtDNA_{del}$	104.73	45.52	108	3	212
Pre-$mtDNA_{del\ ratio}$	50.75%	11.18%	52.4%	21.5%	68.8%
Post-$mtDNA_{del\ ratio}$	47.37%	11.41%	50%	2.7%	65.4%
Diff-$mtDNA_{cn}$	-7.38	48.36	-17	-128	153
Diff-$mtDNA_{del}$	-10.38	44	-13	-133	131
Diff-$mtDNA_{del\ ratio}$	-3.39%	10.43%	-3.7%	-29.7%	24.5%
Symptom checklist					
PCL-pre	55.83	11.62	56	22	77
PCL-post	40.04	17.33	40	5	80
Diff-PCL	-15.79	14.87	-14	-44	17

Note. Negative values in Diff-variables resemble a decline from pre- to post-treatment.
$mtDNA_{cn}$ = number of mitochondrial genomes per monocyte; $mtDNA_{del}$ = mtDNA copies with deletion per monocyte; $mtDNA_{del\ ratio}$ = proportion of mitochondrial genomes with deletion in the total number of mitochondrial genomes ($mtDNA_{del}/mtDNA_{cn}$); PCL = PTSD-Check List for DSM-5

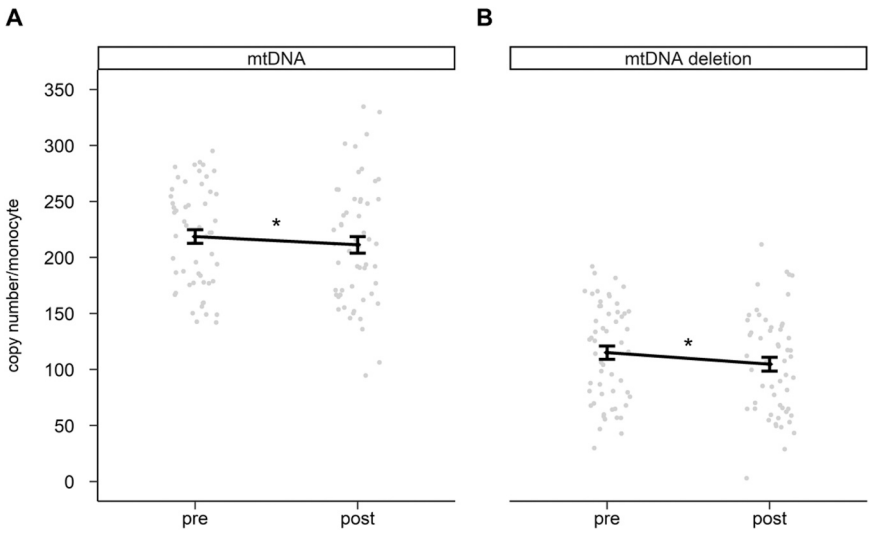

Fig. 1. Total mtDNA copy numbers und mtDNA copies with deletion per monocyte pre and post treatment. **A)** Pre- and post-treatment mtDNA copy number levels are shown. Wilcoxon signed-rank test revealed a significant decline in mtDNA copy number levels after standard inpatient treatment, V = 941, p = .046, d = 0.15, 95% CI [− 0.11, 0.40]. **B)** mtDNA deletion levels also decreased significantly from pre- to post-treatment, V = 1035.5, p = .03, d = 0.23, 95% CI [− 0.03, 0.50]. Scatter plot shows the individual values of the patients. Error bars represent standard error of the mean.

50.75% vs. 47.37%, *SDs* = 11.18% vs. 11.41%), $V = 1059$, $p = .02$, $d = 0.30$, 95% CI [0.05, 0.55] with a medium effect size. The paired t-test for pre- and post-treatment PCL-5 scores revealed a significant decline in symptom-severity (*Ms* = 55.83 vs. 40.04, *SDs* = 11.62 vs. 17.33), $t(54) = 7.878$, $p < .001$, $d = 1.03$, 95% CI [0.71, 1.35].

3.2. Changes in psychotropic medication

Neither Diff-mtDNA$_{cn}$ nor Diff-mtDNA$_{del}$ levels differed significantly between the two categories of changes in psychotropic medication. Due to violations of the assumption of normality, Wilcoxon rank sum tests were used in the statistical analysis.

3.3. Spearman's rank coefficients of correlation

Spearman's rank coefficients of correlation between mitochondrial DNA markers and indicators for symptom-severity are illustrated in Supplementary Table SA3. After adjusting for multiple comparisons, no significant correlations were observed.

3.4. Multiple regression analysis

Controlled for participants age, BMI and changes in psychotropic medication, neither changes in mtDNA$_{cn}$ nor changes in mtDNA$_{del}$ could predict changes in PTSD symptom-severity, (*ps* > 0.05). Results are depicted in Supplemental Table SA3.

4. Discussion

The aim of this study was to determine whether six weeks of inpatient PTSD treatment influence mtDNA$_{cn}$, mtDNA$_{del}$, and the ratio of mtDNA$_{del}$ copies to wild type mtDNA copies (referred to as mtDNA deletion ratio). In addition, we investigated whether changes in mtDNA$_{cn}$ and mtDNA$_{del}$ can predict treatment-response. We analyzed mitochondrial markers in PTSD using isolated cells thus avoiding cell-heterogeneity as a potential confounder of mtDNA$_{cn}$. Group comparisons showed that mean mtDNA$_{cn}$, mtDNA$_{del}$ and mtDNA deletion ratio declined significantly from pre- to post-treatment, indicating that mtDNA can be influenced by external factors such as inpatient treatment (Fig. 1). Changes in mtDNA$_{cn}$ and mtDNA$_{del}$, however, did not correlate with treatment-response. The observation that the mtDNA$_{cn}$ changes over the course of therapy confirms our hypothesis but must nevertheless be viewed critically.

It must be considered that it is not clear which factors exactly contributed to the decline in mtDNA$_{cn}$ levels and mutational load. Since self-reported stress perception and stress hormone levels were not monitored in this study, it may mislead to state that stress was indeed reduced throughout the treatment period. Since mtDNA$_{cn}$ and mutational load can be signs of MAL (Picard et al., 2019), a decrease of those markers can be an indicator for less MAL post-treatment. Especially, considering that although low levels of mtDNA$_{cn}$ may impair mitochondrial function (Jeng et al., 2008), heteroplasmy levels measured via mtDNA deletion ratio also declined, protecting the cell from dysfunctional states (Filograna et al., 2021).

In addition, it is unclear whether the therapeutic setting and the various treatments themselves led to reduced mitochondrial DNA levels or just the change of circumstances and lifestyle during therapy in general. For example, diet changes and vitamin consumption can influence mtDNA$_{cn}$ (Ma et al., 2022; Wu et al., 2019) and thus signs of MAL. Therefore, it cannot be excluded that a different diet during inpatient-treatment influenced variations in mtDNA$_{cn}$ and mutational load. Another factor that can influence mtDNA$_{cn}$ is smoking behavior or the quality of sleep (Ma et al., 2022; Vyas et al., 2020). A study with monozygotic twins and discordant sleeping patterns revealed that sleep duration and efficiency also influenced mtDNA$_{cn}$ levels (Wrede et al., 2015). This could also represent a confounding variable in this study, since it cannot be ruled out that inpatient treatment also influenced personal sleeping patterns. Furthermore, a change in the mtDNA$_{cn}$ could have been triggered by medication. As previous studies have shown, antidepressants can affect mitochondrial function by inhibiting transcription of electron transport chain genes, increasing ROS production, or decreasing endogenous antioxidant levels (as reviewed by Neustadt and Pieczenik, 2008). This was confirmed by Rollins and colleagues (2018) who found decreased mitochondrial electron transport chain enzymatic activity in the brain of patients with SZ and BD taking antipsychotics and antidepressants. When quantifying mitochondrial DNA, they also found that mtDNA$_{cn}$ was significantly increased in SZ and BD compared to controls with no change in mtDNA$_{del}$.

Interestingly, Wang et al. (2017) observed higher levels of mtDNA$_{cn}$ in peripheral blood cells of patients with depression, anxiety, and stress-related disorders during psychotherapy compared to healthy controls. However, there was no significant change in mtDNA$_{cn}$ levels from baseline to post-treatment in their patient cohort. Nevertheless, when looking at interactions with changes in symptom-severity, two of the four symptom scales showed significant correlations with changes in mtDNA$_{cn}$, suggesting a possible connection to treatment-response. Cai et al. (2015) who also investigated mtDNA$_{cn}$ levels in MDD patients compared to a control group found altered levels of mtDNA$_{cn}$ in

peripheral blood cells in MDD. To explore whether stress actively increases $mtDNA_{cn}$, they stressed mice for four weeks and found elevated $mtDNA_{cn}$ in blood and saliva after two and four weeks. When the stressor was discontinued $mtDNA_{cn}$ returned to baseline levels after 8 weeks. In addition, Cai et al. (2015) found a linear relationship between stressful life events and childhood sexual abuse and $mtDNA_{cn}$ in patients with MDD. These findings suggest that stress may lead to elevated $mtDNA_{cn}$ levels and discontinuing the stressor can reverse these effects.

As proposed by Picard and McEwen (2018a, 2018b), increases in $mtDNA_{cn}$ are supposedly related to higher energy demand as well as compensations of high levels of $mtDNA_{mut}$. MtDNA mutations, however, accumulate under cellular stress. It can therefore be assumed that a reduction of stress levels in the context of inpatient treatment can lead to a reduction in $mtDNA_{cn}$ and $mtDNA_{mut\ and/or\ del}$, which also leads to a significant shift in heteroplasmy levels. On the other hand, there are findings showing decreased glucocorticoid levels in patients with PTSD (Yehuda et al., 1990). We would therefore assume an increase in $mtDNA_{cn}$ with a normalization of the HPA axis and a concomitant increase in cortisol levels.

In our study, none of these declines in mitochondrial markers were correlated with symptom-severity or could predict treatment-response in this sample. This stands in contrast to the finding by Wang et al. (2017), who found an association between $mtDNA_{cn}$ and symptom severity as well as an interaction change in $mtDNA_{cn}$ and treatment response in patients with depression, anxiety, and stress related disorders. Our results also contrast with the findings by Humphreys et al. (2020), suggesting that baseline depressive symptoms can precede changes in $mtDNA_{cn}$. Investigating $mtDNA_{cn}$ in granulocytes of male combat veterans with PTSD, Bersani et al. (2016) observed a reduced number of mitochondrial genomes. They found a correlation of $mtDNA_{cn}$ with positive affectivity within PTSD subjects and proposed an inverted-U shaped relationship between PTSD symptom severity and $mtDNA_{cn}$.

Although $mtDNA_{cn}$, $mtDNA_{del}$ and mtDNA deletion ratio were not correlated with treatment-response in our study, the inpatient-treatment was significantly associated with mitochondrial markers as well as symptom severity. Since this was the first study to use both, $mtDNA_{cn}$ as well as $mtDNA_{del}$ as predictors for treatment response, further research needs to replicate these findings.

Critically, it should be noted that no control group was examined, since the focus of this study was primarily on examining molecular markers during therapy and precludes comparisons with healthy controls who received no treatment or lifestyle changes impossible.

As elaborated above, mitochondrial function and dysfunction are increasingly implicated in mental disorders. Although there are diverging findings regarding the direction (Chang et al., 2015; Chung et al., 2022), $mtDNA_{cn}$ levels and heteroplasmy levels are related to mental disorders as well. Monitoring copy number levels and mutational load among other indicators like childhood sexual abuse and severe life events could therefore offer an opportunity to predict the risk of developing a mental disorder. It is evident that reducing $mtDNA_{mut}$ levels below threshold, an improvement in illness is accomplished (Bacman et al., 2013; Hayashi et al., 1991; Smith and Lightowlers, 2011). In recent years, therapeutic manipulations that can shift heteroplasmy levels to a more functional mitochondrial state have been discussed (Jackson et al., 2020; Pereira and Moraes, 2017). One therapeutic strategy that was discussed in this context was exercise, but current results are controversial (Murphy et al., 2008; Taivassalo et al., 1999, 2006). It was also discussed that ketogenic supplements could shift heteroplasmy towards an increase in wild type mtDNA (Santra et al., 2004) but most strategies to shift heteroplasmy that have been discussed are in vitro methods or therapeutic approaches that must yet find their way into clinical practice (Jackson et al., 2020). Our study shows promising results to reduce $mtDNA_{del}$ levels and mtDNA deletion ratio for mental disorders by inpatient-treatment including various forms of psychotherapy. However, it is important to note that our study is only indicative of the common mtDNA deletion in association with PTSD. A stress-induced cumulative load of rare SNPs and mtDNA deletions that could lead to PTSD cannot be ruled out but cannot be reflected here. Furthermore, mitochondrial function has not been measured. More recent studies measure not only $mtDNA_{cn}$ but also the activity of mitochondrial enzymes such as citrate synthase, cytochrome c oxidase and succinate dehydrogenase using kinetic spectrophotometric assays (Behnke et al., 2022; Fernstrom et al., 2021a; Gumpp et al., 2021, 2022, 2020).

Although cognitive-behavioral therapy and exposure-based therapy included in inpatient-treatment have been proven effective for treating PTSD (Jonas et al., 2013; Watts et al., 2013), there are still patients who do not respond to them. Therefore, it is crucial to understand the mechanisms that contribute to a reduction of copy number and heteroplasmy levels during (psycho-)therapy to target a shift in heteroplasmy specifically as a possibility of treatment augmentation. Moreover, it is important to understand to which degree mitochondrial (dys-)function can influence symptom-severity and treatment-response, to adjust treatment for those whose prognosis suggests that they benefit less from the treatment. Although no causal conclusion can be made due to the study design, this study shows that inpatient PTSD treatment can reduce signs of MAL, possibly enhancing mitochondrial function and consequently, mental health. Although further research still must evaluate whether $mtDNA_{cn}$ (and other mitochondrial markers such as heteroplasmy) are useful biomarkers, the results of this study support the idea that $mtDNA_{cn}$ and $mtDNA_{del}$ can be signs of MAL and that a less stressful environment or a general change of external factors can reduce MAL, which is promising for the implementation of $mtDNA_{cn}$ as a biomarker for mental illness. Future studies could also focus on examining cell-free DNA (cfDNA) and cell-free mtDNA (cf-mtDNA) as we were recently able to show that both acute psychosocial and acute physical stress lead to increased values of these markers in the periphery (Hummel et al., 2018; Trumpff et al., 2021). Fernstrom et al. (2021b) also recently showed changes in cell-free mtDNA in MDD patients compared to healthy controls and an association between mood stabilizer intake and cf-mtDNA levels within the MDD group.

To our knowledge, this is the first study to measure changes in $mtDNA_{cn}$, $mtDNA_{del}$ and heteroplasmy levels in an isolated tissue over six weeks of inpatient-treatment in a cohort of PTSD patients. Other studies either investigated $mtDNA_{cn}$ in heterogenous disorder samples or different mental disorders, used whole-blood samples or did not consider the change in markers over time. We are aware that the significant differences in the overall mtDNA population are minimal when comparing existing cellular mtDNA before and after therapy. However, with a complex phenotype such as PTSD, this is not surprising. We continue to believe that changes in mtDNA counts is just one of many pieces of the molecular puzzle that contribute to behavioral changes and differences in well-being. In this context, it would certainly be interesting to investigate mtDNA in other psychiatric phenotypes with clinical pictures that are phenotypically at opposite ends, such as burnout, depression, or attention deficit hyperactivity disorder.

In conclusion, our study shows that a change in environmental factors can reduce mitochondrial markers such as $mtDNA_{cn}$, total number $mtDNA_{del}$ and therefore the ratio from wild type to mtDNA with a large deletion. This indicates that signs of MAL were reduced over time, with potential beneficial effects on mental health. These changes did not correlate with symptom-severity and treatment-response in this sample, leaving room for future studies to explore the underlying mechanisms further.

Future studies should therefore examine more closely which specific lifestyle and treatment factors contribute to the reduction in mitochondrial markers as identified here, and include investigation of other signs of MAL, such as ROS production and enzyme activity in mitochondria, as well as cortisol-measurements to observe changes in stress-levels. Taken into consideration the pilot-study character of our study, the results are already very promising, encouraging further research in

this field.

Conflicts of interest

All authors declare that they have no conflicts of interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105993.

References

Anglin, R.E., Garside, S.L., Tarnopolsky, M.A., Mazurek, M.F., Rosebush, P.I., 2012. The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J. Clin. Psychiatry 73, 506–512.
APA, A.P.A., 2013. Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition). American Psychiatric Association Publishing.
Atwoli, L., Stein, D.J., Koenen, K.C., McLaughlin, K.A., 2015. Epidemiology of posttraumatic stress disorder: prevalence, correlates and consequences. Curr. Opin. Psychiatr. 28, 307–311.
Bacman, S.R., Williams, S.L., Pinto, M., Peralta, S., Moraes, C.T., 2013. Specific elimination of mutant mitochondrial genomes in patient-derived cells by mitoTALENs. Nat. Med. 19, 1111–1113.
Behnke, A., Gumpp, A.M., Rojas, R., Sanger, T., Lutz-Bonengel, S., Moser, D., Schelling, G., Krumbholz, A., Kolassa, I.T., 2022. Circulating inflammatory markers, cell-free mitochondrial DNA, cortisol, endocannabinoids, and N-acylethanolamines in female depressed outpatients. World J. Biol. Psychiatry 1–12.
Bersani, F.S., Morley, C., Lindqvist, D., Epel, E.S., Picard, M., Yehuda, R., Flory, J., Bierer, L.M., Makotkine, I., Abu-Amara, D., Coy, M., Reus, V.I., Lin, J., Blackburn, E. H., Marmar, C., Wolkowitz, O.M., Mellon, S.H., 2016. Mitochondrial DNA copy number is reduced in male combat veterans with PTSD. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 64, 10–17.
Bremner, D., Vermetten, E., Kelley, M.E., 2007. Cortisol, dehydroepiandrosterone, and estradiol measured over 24 h in women with childhood sexual abuse-related posttraumatic stress disorder. J. Nerv. Ment. Dis. 195, 919–927.
Cai, N., Li, Y.H., Chang, S., Liang, J.Q., Lin, C.Y., Zhang, X.F., Liang, L., Hu, J.C., Chan, W., Kendler, K.S., Malinauskas, T., Huang, G.J., Li, Q.B., Mott, R., Flint, J., 2015. Genetic control over mtDNA and its relationship to major depressive disorder. Curr. Biol. 25, 3170–3177.
Castellani, C.A., Longchamps, R.J., Sun, J., Guallar, E., Arking, D.E., 2020. Thinking outside the nucleus: mitochondrial DNA copy number in health and disease. Mitochondrion 53, 214–223.
Chang, C.C., Jou, S.H., Lin, T.T., Lai, T.J., Liu, C.S., 2015. Mitochondria DNA change and oxidative damage in clinically stable patients with major depressive disorder. PLoS One 10.
Chung, J.K., Ahn, Y.M., Kim, S.A., Joo, E.J., 2022. Differences in mitochondrial DNA copy number between patients with bipolar I and II disorders. J. Psychiatr. Res. 145, 325–333.
Cohen, J., 1992. A power primer. Psychol. Bull. 112, 155–159.
Daniels, T.E., Olsen, E.M., Tyrka, A.R., 2020. Stress and psychiatric disorders: the role of mitochondria. Annu. Rev. Clin. Psychol. 16, 165–186.
Fernstrom, J., Mellon, S.H., McGill, M.A., Picard, M., Reus, V.I., Hough, C.M., Lin, J., Epel, E.S., Wolkowitz, O.M., Lindqvist, D., 2021a. Blood-based mitochondrial respiratory chain function in major depression. Transl. Psychiatry 11.
Fernstrom, J., Ohlsson, L., Asp, M., Lavant, E., Holck, A., Grudet, C., Westrin, A., Lindqvist, D., 2021b. Plasma circulating cell-free mitochondrial DNA in depressive disorders. PLoS One 16, e0259591.
Filograna, R., Mennuni, M., Alsina, D., Larsson, N.G., 2021. Mitochondrial DNA copy number in human disease: the more the better? FEBS Lett. 595, 976–1002.
Flaquer, A., Baumbach, C., Ladwig, K.H., Kriebel, J., Waldenberger, M., Grallert, H., Baumert, J., Meitinger, T., Kruse, J., Peters, A., Emeny, R., Strauch, K., 2015. Mitochondrial genetic variants identified to be associated with posttraumatic stress disorder. Transl. Psychiatry 5.
Gelernter, J., Sun, N., Polimanti, R., Pietrzak, R., Levey, D.F., Bryois, J., Lu, Q., Hu, Y., Li, B., Radhakrishnan, K., Aslan, M., Cheung, K.H., Li, Y., Rajeevan, N., Sayward, F., Harrington, K., Chen, Q., Cho, K., Pyarajan, S., Sullivan, P.F., Quaden, R., Shi, Y., Hunter-Zinck, H., Gaziano, J.M., Concato, J., Zhao, H., Stein, M.B., Department of Veterans Affairs Cooperative Studies, P., Million Veteran, P., 2019. Genome-wide association study of post-traumatic stress disorder reexperiencing symptoms in >165,000 US veterans. Nat. Neurosci. 22, 1394–1401.
Gumpp, A.M., Boeck, C., Behnke, A., Bach, A.M., Ramo-Fernandez, L., Welzb, T., Gundel, H., Kolassa, I.T., Karabatsiakisa, A., 2020. Childhood maltreatment is associated with changes in mitochondrial bioenergetics in maternal, but not in neonatal immune cells. Proc. Natl. Acad. Sci. USA 117, 24778–24784.
Gumpp, A.M., Behnke, A., Bach, A.M., Piller, S., Boeck, C., Rojas, R., Kolassa, I.T., 2021. Mitochondrial bioenergetics in leukocytes and oxidative stress in blood serum of mild to moderately depressed women. Mitochondrion 58, 14–23.
Gumpp, A.M., Behnke, A., Ramo-Fernandez, L., Radermacher, P., Gundel, H., Ziegenhain, U., Karabatsiakis, A., Kolassa, I.T., 2022. Investigating mitochondrial bioenergetics in peripheral blood mononuclear cells of women with childhood maltreatment from post-parturition period to one-year follow-up. Psychol. Med.
Hayashi, J.I., Ohta, S., Kikuchi, A., Takemitsu, M., Goto, Y., Nonaka, I., 1991. Introduction of disease-related mitochondrial-DNA deletions into hela-cells lacking mitochondrial-DNA results in mitochondrial dysfunction. Proc. Natl. Acad. Sci. USA 88, 10614–10618.
Hebert-Chatelain, E., Desprez, T., Serrat, R., Bellocchio, L., Soria-Gomez, E., Busquets-Garcia, A., Zottola, A.C.P., Delamarre, A., Cannich, A., Vincent, P., Varilh, M., Robin, L.M., Terral, G., Garcia-Fernandez, M.D., Colavita, M., Mazier, W., Drago, F., Puente, N., Reguero, L., Elezgarai, I., Dupuy, J.W., Cota, D., Lopez-Rodriguez, M.L., Barreda-Gomez, G., Massa, F., Grandes, P., Benard, G., Marsicano, G., 2016. A cannabinoid link between mitochondria and memory. Nature 539, 555-+.
Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.
Hummel, E.M., Hessas, E., Muller, S., Beiter, T., Fisch, M., Eibl, A., Wolf, O.T., Giebel, B., Platen, P., Kumsta, R., Moser, D.A., 2018. Cell-free DNA release under psychosocial and physical stress conditions. Transl. Psychiatry 8, 236.
Humphreys, K.L., Sisk, L.M., Manczak, E.M., Lin, J., Gotlib, I.H., 2020. Depressive symptoms predict change in telomere length and mitochondrial DNA copy number across adolescence. J. Am. Acad. Child Adolesc. Psychiatry 59, 1364.
Jackson, C.B., Turnbull, D.M., Minczuk, M., Gammage, P.A., 2020. Therapeutic manipulation of mtDNA heteroplasmy: a shifting perspective. Trends Mol. Med. 26, 698–709.
Jeng, J.Y., Yeh, T.S., Lee, J.W., Lin, S.H., Fong, T.H., Hsieh, R.H., 2008. Maintenance of mitochondrial DNA copy number and expression are essential for preservation of mitochondrial function and cell growth. J. Cell Biochem. 103, 347–357.
Jonas, D.E., Cusack, K., Forneris, C.A., Wilkins, T.M., Sonis, J., Middleton, J.C., Feltner, C., Meredith, D., Cavanaugh, J., Brownley, K.A., Olmsted, K.R., Greenblatt, A., Weil, A., Gaynes, B.N., 2013. Psychological and Pharmacological Treatments for Adults With Posttraumatic Stress Disorder (PTSD). Rockville (MD).
Jou, S.H., Chiu, N.Y., Liu, C.S., 2009. Mitochondrial dysfunction and psychiatric disorders. Chang Gung Med. J. 32, 370–379.
Kasahara, T., Kato, T., 2018. What can mitochondrial DNA analysis tell us about mood disorders? Biol. Psychiatry 83, 731–738.
Kim, T.D., Lee, S., Yoon, S., 2020. Inflammation in post-traumatic stress disorder (PTSD): a review of potential correlates of PTSD with a neurological perspective. Antioxidants 9.
Klaassens, E.R., Giltay, E.J., Cuijpers, P., van Veen, T., Zitman, F.G., 2012. Adulthood trauma and HPA-axis functioning in healthy subjects and PTSD patients: a meta-analysis. Psychoneuroendocrinology 37, 317–331.
Kruger-Gottschalk, A., Knaevelsrud, C., Rau, H., Dyer, A., Schafer, I., Schellong, J., Ehring, T., 2017. The German version of the Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5): psychometric properties and diagnostic utility. BMC Psychiatry 17, 379.
Kumsta, R., 2019. The role of epigenetics for understanding mental health difficulties and its implications for psychotherapy research. Psychol. Psychother. 92, 190–207.
Kuznetsov, A.V., Kehrer, I., Kozlov, A.V., Haller, M., Redl, H., Hermann, M., Grimm, M., Troppmair, J., 2011. Mitochondrial ROS production under cellular stress: comparison of different detection methods. Anal. Bioanal. Chem. 400, 2383–2390.
Lanius, R., Olff, M., 2017. The neurobiology of PTSD. Eur. J. Psychotraumatol. 8, 1314165.
Lee, H.C., Chang, C.M., Chi, C.W., 2010. Somatic mutations of mitochondrial DNA in aging and cancer progression. Ageing Res. Rev. 9 (Suppl 1), S47–S58.
Logue, M.W., Miller, M.W., Wolf, E.J., Huber, B.R., Morrison, F.G., Zhou, Z., Zheng, Y., Smith, A.K., Daskalakis, N.P., Ratanatharathorn, A., Uddin, M., Nievergelt, C.M., Ashley-Koch, A.E., Baker, D.G., Beckham, J.C., Garrett, M.E., Boks, M.P., Geuze, E., Grant, G.A., Hauser, M.A., Kessler, R.C., Kimbrel, N.A., Maihofer, A.X., Marx, C.E., Qin, X.J., Risbrough, V.B., Rutten, B.P.F., Stein, M.B., Ursano, R.J., Vermetten, E., Vinkers, C.H., Ware, E.B., Stone, A., Schichman, S.A., McGlinchey, R.E., Milberg, W. P., Hayes, J.P., Verfaellie, M., Traumatic Stress Brain Study, G., 2020. An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. Clin. Epigenet. 12, 46.
Ma, J.T., Liu, X., Zhang, Y.K., Cheng, H.N., Gao, W.C., Lai, C.Q., Gabriel, S., Gupta, N., Vasan, R.S., Levy, D., Liu, C.Y., 2022. Diet quality scores are positively associated with whole blood-derived mitochondrial DNA copy number in the Framingham heart study. J. Nutr. 152, 690–697.
Meewisse, M.L., Reitsma, J.B., de Vries, G.J., Gersons, B.P., Olff, M., 2007. Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. Br. J. Psychiatry 191, 387–392.
Miller, B., Bennett, J.P., 2010. The locus ceruleus contains mitochondrial DNA deletions in Alzheimer's Disease. Mitochondrion 10, 239–240.
Mondelli, V., Vernon, A.C., 2019. From early adversities to immune activation in psychiatric disorders: the role of the sympathetic nervous system. Clin. Exp. Immunol. 197, 319–328.
Moser, D.A., Muller, S., Hummel, E.M., Limberg, A.S., Dieckmann, L., Frach, L., Pakusch, J., Flasbeck, V., Brune, M., Beygo, J., Klein-Hitpass, L., Kumsta, R., 2020. Targeted bisulfite sequencing: a novel tool for the assessment of DNA methylation

with high sensitivity and increased coverage. Psychoneuroendocrinology 120, 104784.
Murphy, J.L., Blakely, E.L., Schaefer, A.M., He, L., Wyrick, P., Haller, R.G., Taylor, R.W., Turnbull, D.M., Taivassalo, T., 2008. Resistance training in patients with single, large-scale deletions of mitochondrial DNA. Brain 131, 2832–2840.
Neustadt, J., Pieczenik, S.R., 2008. Medication-induced mitochondrial damage and disease. Mol. Nutr. Food Res. 52, 780–788.
Neylan, T.C., Brunet, A., Pole, N., Best, S.R., Metzler, T.J., Yehuda, R., Marmar, C.R., 2005. PTSD symptoms predict waking salivary cortisol levels in police officers. Psychoneuroendocrinology 30, 373–381.
Nicod, J., Wagner, S., Vonberg, F., Bhomra, A., Schlicht, K.F., Tadic, A., Mott, R., Lieb, K., Flint, J., 2016. The amount of mitochondrial DNA in blood reflects the course of a depressive episode. Biol. Psychiatry 80, E41–E42.
Olff, M., 2017. Sex and gender differences in post-traumatic stress disorder: an update. Eur. J. Psychotraumatol. 8.
Pereira, C.V., Moraes, C.T., 2017. Current strategies towards therapeutic manipulation of mtDNA heteroplasmy. Front. Biosci. -Landmark 22, 991–1010.
Phillips, N.R., Simpkins, J.W., Roby, R.K., 2014a. Mitochondrial DNA deletions in Alzheimer's brains: a review. Alzheimers Dement. 10, 393–400.
Phillips, N.R., Sprouse, M.L., Roby, R.K., 2014b. Simultaneous quantification of mitochondrial DNA copy number and deletion ratio: a multiplex real-time PCR assay. Sci. Rep. 4.
Picard, M., McEwen, B.S., 2018a. Psychological stress and mitochondria: a conceptual framework. Psychosom. Med. 80, 126–140.
Picard, M., McEwen, B.S., 2018b. Psychological stress and mitochondria: a systematic review. Psychosom. Med. 80, 141–153.
Picard, M., Juster, R.P., McEwen, B.S., 2014. Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. Nat. Rev. Endocrinol. 10, 303–310.
Picard, M., Wallace, D.C., Burelle, Y., 2016. The rise of mitochondria in medicine. Mitochondrion 30, 105–116.
Picard, M., McEwen, B.S., Epel, E.S., Sandi, C., 2018. An energetic view of stress: focus on mitochondria. Front. Neuroendocrinol. 49, 72–85.
Picard, M., Trumpff, C., Burelle, Y., 2019. Mitochondrial psychobiology: foundations and applications. Curr. Opin. Behav. Sci. 28, 142–151.
Preston, G., Kirdar, F., Kozicz, T., 2018. The role of suboptimal mitochondrial function in vulnerability to post-traumatic stress disorder. J. Inherit. Metab. Dis. 41, 585–596.
Psarra, A.M.G., Sekeris, C.E., 2011. Glucocorticoids induce mitochondrial gene transcription in HepG2 cells Role of the mitochondrial glucocorticoid receptor. Biochim. Biophys. Acta Mol. Cell Res. 1813, 1814–1821.
Psarra, A.M.G., Solakidi, S., Sekeris, C.E., 2006a. The mitochondrion as a primary site of action of regulatory agents involved in neuroimmunomodulation. Neuroendocr. Immune Cross 1088, 12–22.
Psarra, A.M.G., Solakidi, S., Sekeris, C.E., 2006b. The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells. Mol. Cell. Endocrinol. 246, 21–33.
Reguly, B., Jakupciak, J.P., Parr, R.L., 2010. 3.4 kb mitochondrial genome deletion serves as a surrogate predictive biomarker for prostate cancer in histopathologically benign biopsy cores. Can. Urol. Assoc. J. 4, E118–E122.
Roberts, R.C., 2017. Postmortem studies on mitochondria in schizophrenia. Schizophr. Res. 187, 17–25.
Rohleder, N., Joksimovic, L., Wolf, J.M., Kirschbaum, C., 2004. Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. Biol. Psychiatry 55, 745–751.
Rollins, B.L., Morgan, L., Hjelm, B.E., Sequeira, A., Schatzberg, A.F., Barchas, J.D., Lee, F. S., Myers, R.M., Watson, S.J., Akil, H., Potkin, S.G., Bunney, W.E., Vawter, M.P., 2018. Mitochondrial complex I deficiency in schizophrenia and bipolar disorder and medication influence. Mol. Neuropsychiatry 3, 157–169.
Santra, S., Gilkerson, R.W., Davidson, M., Schon, E.A., 2004. Ketogenic treatment reduces deleted mitochondrial DNAs in cultured human cells. Ann. Neurol. 56, 662–669.
Shao, L., Martin, M.V., Watson, S.J., Schatzberg, A., Akil, H., Myers, R.M., Jones, E.G., Bunney, W.E., Vawter, M.P., 2008. Mitochondrial involvement in psychiatric disorders. Ann. Med. 40, 281–295.
Smith, A.K., Ratanatharathorn, A., Maihofer, A.X., Naviaux, R.K., Aiello, A.E., Amstadter, A.B., Ashley-Koch, A.E., Baker, D.G., Beckham, J.C., Boks, M.P., Bromet, E., Dennis, M., Galea, S., Garrett, M.E., Geuze, E., Guffanti, G., Hauser, M.A., Katrinli, S., Kilaru, V., Kessler, R.C., Kimbrel, N.A., Koenen, K.C., Kuan, P.F., Li, K., Logue, M.W., Lori, A., Luft, B.J., Miller, M.W., Naviaux, J.C., Nugent, N.R., Qin, X., Ressler, K.J., Risbrough, V.B., Rutten, B.P.F., Stein, M.B., Ursano, R.J., Vermetten, E., Vinkers, C.H., Wang, L., Youssef, N.A., Consortium, I.N.C., Workgroup, V.A.M.-A.M., Workgroup, P.P.E., Uddin, M., Nievergelt, C.M., 2020. Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies methylation changes in AHRR. Nat. Commun. 11, 5965.
Smith, P.M., Lightowlers, R.N., 2011. Altering the balance between healthy and mutated mitochondrial DNA. J. Inherit. Metab. Dis. 34, 309–313.
Speer, K.E., Semple, S., Naumovski, N., D'Cunha, N.M., McKune, A.J., 2019. HPA axis function and diurnal cortisol in post-traumatic stress disorder: a systematic review. Neurobiol. Stress 11, 100180.
Stein, M.B., Levey, D.F., Cheng, Z., Wendt, F.R., Harrington, K., Pathak, G.A., Cho, K., Quaden, R., Radhakrishnan, K., Girgenti, M.J., Ho, Y.A., Posner, D., Aslan, M., Duman, R.S., Zhao, H., Department of Veterans Affairs Cooperative Studies, P., Program, V.A.M.V., Polimanti, R., Concato, J., Gelernter, J., 2021. Genome-wide association analyses of post-traumatic stress disorder and its symptom subdomains in the Million Veteran Program. Nat. Genet. 53, 174–184.
Taivassalo, T., Fu, K., Johns, T., Arnold, D., Karpati, G., Shoubridge, E.A., 1999. Gene shifting: a novel therapy for mitochondrial myopathy. Hum. Mol. Genet. 8, 1047–1052.
Taivassalo, T., Gardner, J.L., Taylor, R.W., Schaefer, A.M., Newman, J., Barron, M.J., Haller, R.G., Turnbull, D.M., 2006. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. Brain 129, 3391–3401.
Trumpff, C., Michelson, J., Lagranha, C.J., Taleon, V., Karan, K.R., Sturm, G., Lindqvist, D., Fernstrom, J., Moser, D., Kaufman, B.A., Picard, M., 2021. Stress and circulating cell-free mitochondrial DNA: a systematic review of human studies, physiological considerations, and technical recommendations. Mitochondrion 59, 225–245.
Vyas, C.M., Ogata, S., Reynolds 3rd, C.F., Mischoulon, D., Chang, G., Cook, N.R., Manson, J.E., Crous-Bou, M., De Vivo, I., Okereke, O.I., 2020. Lifestyle and behavioral factors and mitochondrial DNA copy number in a diverse cohort of mid-life and older adults. PLoS One 15, e0237235.
Wallace, D.C., 1992. Diseases of the mitochondrial-DNA. Annu. Rev. Biochem. 61, 1175–1212.
Wallace, D.C., 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. 39, 359–407.
Wang, X., Sundquist, K., Rastkhani, H., Palmer, K., Memon, A.A., Sundquist, J., 2017. Association of mitochondrial DNA in peripheral blood with depression, anxiety and stress- and adjustment disorders in primary health care patients. Eur. Neuropsychopharmacol. 27, 751–758.
Watts, B.V., Schnurr, P.P., Mayo, L., Young-Xu, Y., Weeks, W.B., Friedman, M.J., 2013. Meta-analysis of the efficacy of treatments for posttraumatic stress disorder. J. Clin. Psychiatry 74, e541–e550.
Weathers, F.W., Keane, T.M., Palmieri, P.A., Marx, B.P., Schnurr, P.P., 2013. PTSD Checklist for DSM-5 (PCL-5). PTSD: National Center for PTSD 2013;10(4).
Wrede, J.E., Mengel-From, J., Buchwald, D., Vitiello, M.V., Bamshad, M., Noonan, C., Christiansen, L., Christensen, K., Watson, N.F., 2015. Mitochondrial DNA copy number in sleep duration discordant monozygotic twins. Sleep 38, 1655–U1153.
Wu, S.W., Li, X., Meng, S.S., Fung, T., Chan, A.T., Liang, G.Y., Giovannucci, E., De Vivo, I., Lee, J.H., Nan, H.M., 2019. Fruit and vegetable consumption, cigarette smoke, and leukocyte mitochondrial DNA copy number. Am. J. Clin. Nutr. 109, 424–432.
Xie, P., Kranzler, H.R., Yang, C., Zhao, H., Farrer, L.A., Gelernter, J., 2013. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. Biol. Psychiatry 74, 656–663.
Yehuda, R., 2005. Neuroendocrine aspects of PTSD. Handb. Exp. Pharmacol. 371–403.
Yehuda, R., Southwick, S.M., Nussbaum, G., Wahby, V., Giller Jr., E.L., Mason, J.W., 1990. Low urinary cortisol excretion in patients with posttraumatic stress disorder. J. Nerv. Ment. Dis. 178, 366–369.
Yehuda, R., Teicher, M.H., Trestman, R.L., Levengood, R.A., Siever, L.J., 1996. Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. Biol. Psychiatry 40, 79–88.

Supplemental material

Table SA1. Sample characteristics.

Diagnose (ICD 10)	**n**	**%**
PTSD (F43.1)	55	100
additional MDD (F33.1)	52	94.5
Comorbidities		
no other comorbidities	18	32.7
1 comorbidity	12	21.8
2 comorbidities	9	16.4
3 comorbidities	7	12.7
>3 comorbidities	9	16.4
Diagnosis of comorbidities (ICD 10)		
other mental disorders (F00-F99)	30	54.5
Endocrine, nutritional, and metabolic diseases (E00-E99)	14	25.5
Diseases of the nervous system (G00-G99)	9	16.4
Diseases of the circulatory system (I00-I99)	7	12.7
Diseases of the respiratory system (J00-J99)	4	7.4
Diseases of the musculoskeletal system (M00-M99)	9	16.4
Medication	**n**	**%**
No medication	6	10.9
Non psychotropic drug	7	12.7
Psychotropic drug	42	76.4
Change in psychotropic medication		
no	23	41.8
yes	32	58.2

Table SA2. Primer binding sites and binding sequence.

Primer Name	Binding Site Position	Sequence (5‘ – 3‘)
For_mtMinArc 50nM	mt 16,528 – 16,548	CTAAATAGCCCACACGTTCCC
Rev_mtMinArc 50nM	mt 23 – 42	AGAGCTCCCGTGAGTGGTTA
HEX-mtMin-BHQ1 Probe 250nM	HEX-CAT[+C]AC[+G]AT[+G][+G]A[+T]CA[+C]AGGT-BHQ1	
For_mtMajArc 50nM	mt 10,912 – 10,931	CTGTTCCCCAACCTTTTCCT
Rev_mtMajArc 50nM	mt 10,975 – 10,994	CCATGATTGTGAGGGGTAGG
TexasRed-mtMaj-BHQ2 Probe 250nM	TxRed-GACC[+C]C[+C]TAA[+C]AACCCCC-BHQ2	
For_B2M 1250nM	Chr15 15,798,932 – 15,798,958	GCTGGGTAGCTCTAAACAATGTATTCA
Rev_B2M 1250nM	Chr15 15,798,999 – 15,799,026	CCATGTACTAACAAATGTCTAAAATGGT
B2M Probe 250nM	FAM-CAG[+C]CT[+A]TT[+C]TG[+C]CAGCCT-BHQ1	

*BHQ stands for black hole quencher®; [+] for locked nucleic acids (LNA);

Multiplex-PCRs were performed in triplicates in a 10 µl final volume containing 5ng human genomic DNA, 5 µl SsoAdvanced Universal Probe Supermix (Biorad, Hercules; USA), and primers and probes as indicated in table SA2. Cycling conditions on a CFX384 Cycler (Biorad, Hercules; USA) were 94°C for 2 min followed by 35 cycles 94°C for 10 seconds and 55°C for 1 minute. Inter-run calibration and data normalization was performed using CFX Maestro 2.3 Software (Biorad, Hercules; USA). MtDNA copy number was quantified using the $\Delta\Delta$CT method compared to known mtDNA copy numbers of the lab intern cell lines HEK293 (human embryonic kidney 293 cell line), THP-1 (human monocytic cell line), U251 (human glioblastoma cell line).

Table SA 3. Spearman's rank coefficients of correlation for all mtDNA markers and symptom severity

Spearman's rank coefficients of correlation	**PCL-pre**	**PCL-post**	**Diff-PCL**
Pre-mtDNA$_{cn}$	-.37	-.31	-.07
Post-MtDNA$_{cn}$	-.23	-.16	.02
Pre-mtDNA$_{del}$	-.39	-.26	-.01
Post-mtDNA$_{del}$	-.20	-.14	.01
Pre-mtDNA$_{del\ ratio}$	-.36	-.18	.04
Post-mtDNA$_{del\ ratio}$	-.22	-.18	-.00
Diff-mtDNA$_{cn}$	.08	.03	.00
Diff-mtDNA$_{del}$	.12	.02	-.05
Diff-mtDNA$_{del\ ratio}$	.12	-.05	-.11

Note. Respective *p*-values were > .05.

Table SA4. Regression analysis of changes in PCL-5 scores (Diff-PCL)

Predictor variable	***b***	***SE b***	**β**	***t***	***p***
Diff-mtDNA$_{cn}$	0.23	0.13	.73	1.74	.09
Diff-mtDNA$_{del}$	-0.22	0.14	-.66	-1.57	.12
Age	-0.05	0.18	-.04	-0.25	.80
BMI	0.03	0.29	.01	0.10	.92
Medication change	-0.93	4.20	-.03	-0.22	.83

Note. R^2 = .06, adj. R^2 = -.04, p = .68, N = 55.