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Increased Rate of Unique Mitochondrial DNA Deletion Breakpoints in Young Adults With Early-Life Stress

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ABSTRACT

BACKGROUND: Mounting evidence suggests that mitochondria respond to psychosocial stress. Recent studies suggest mitochondrial DNA (mtDNA) deletions may be increased in some psychiatric disorders, but no studies have examined early-life stress (ELS) and mtDNA deletions. In this study, we assessed mtDNA deletions in peripheral blood mononuclear cells of medically healthy young adults with and without ELS.

METHODS: Participants (n = 181; 69% female), ages 18 to 40 years, were recruited from the community. Participants with ELS (n = 108) had moderate to severe childhood maltreatment; 83 also had parental loss, and 59 had psychiatric disorders. Participants in the control group (n = 73) had no maltreatment, parental loss, or psychiatric disorders. Standardized interviews and self-report measures assessed demographic variables, stress, and mental health. mtDNA from peripheral blood mononuclear cells was amplified via long-range polymerase chain reaction; mtDNA deletions were quantified via Seq-Well, next-generation sequencing, and the Splice-Break pipeline. Linear regression models were used to assess relationships of mtDNA deletion metrics with ELS, adult stressors, psychiatric disorders, and demographics.

RESULTS: Participants with ELS had significantly greater rates of unique mtDNA deletion breakpoints per 10,000 coverage than participants without ELS ($p < .001$), correcting for age, sex, and sequencing depth. Cumulative mtDNA deletion read percentage was not significantly different between groups. Psychiatric disorders and adult stressors were associated with greater unique mtDNA deletion breakpoints ($ps < .05$) but did not account for associations of ELS with mtDNA deletions.

CONCLUSIONS: The increased number of unique mtDNA deletion breakpoints in participants with ELS suggests that mitochondrial genomes undergo observable alterations in the context of early stress. Future studies will examine mtDNA deletions with metabolic health measures.

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Early-life stress (ELS) is a major risk factor for a range of health conditions and premature mortality (1–5). Robust evidence from diverse biomedical disciplines has demonstrated the effects of ELS on the structure and function of organ systems and in constituent cellular and molecular processes (6,7). Biological effects of early adversity are wide ranging and include epigenetic modifications that alter the expression of stress hormones and neurotransmitters (8), the selective activation and suppression of inflammatory cascades (9,10), and accelerated cellular aging via telomere length and mitochondrial DNA (mtDNA) copy number (11,12). However, the mechanisms that underlie the relationship between childhood adversity and disease development are multifactorial and are not fully understood. In recent years, increasing interest has focused on alterations observed in mitochondria in response to stress, partly due to their well-established contributions to health and disease and their pivotal functions in cellular energy, inflammation, and cell signaling.

Mitochondria are organelles that play a central role in producing cellular energy in the form of adenosine triphosphate (ATP). Cells contain hundreds to thousands of mitochondria, which possess their own maternally inherited genome, mtDNA, which is separate and distinct from nuclear DNA. Each mitochondria contains multiple copies of the ~16.5-kb circular, double-stranded genome (13,14). Genetic disorders ascribed to mtDNA mutations, including mtDNA deletions, affect various organ systems with a propensity for muscular and neurological involvement due to high-energy demands and high mitochondrial copy number in these tissues. Mitochondrial disorders often include neuropsychiatric symptoms including developmental delay, dementia, and depression (15,16).

A growing body of evidence suggests a role for mitochondria in the deleterious effects of childhood adversity. In the acute physiological response to stress, mitochondria generate cellular energy, produce steroid hormones (including cortisol), and activate inflammatory and cell-signaling pathways (17). Severe or

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chronic stress early in life appears to alter mitochondrial structure and function, particularly with respect to mtDNA (18–23). Our group and others have observed increased mtDNA copy number in adolescents (24) and preschool-age children (25,26) exposed to ELS. This increase in mtDNA copy number is not universally observed in stress because it has been found to be decreased in veterans with posttraumatic stress disorder (27) and in peripartum mothers (28). Additionally, circulating levels of cell-free mtDNA have been observed to fluctuate dynamically in response to stressors (29–31), indicating that mtDNA may be released into peripheral biofluids through apoptosis, necrosis, or by active extrusion from cells in response to stress (31). Cell-free mtDNA can function as damage-associated molecular proteins, eliciting a characteristic immune response (similar to that of bacterial DNA) that may contribute to stress-associated physiological changes and negatively affect health outcomes (32).

Rates of structural variants in mtDNA, such as large deletions, also appear to be influenced by cellular stress and have been observed to be increased with greater reactive oxygen species and advanced age. A range of experimental work connects mitochondrial dysfunction and ELS-associated pathologies such as vascular disease, chronic inflammation, and neurodegenerative disorders, and mtDNA deletions are considered a potential disease marker for such processes (33,34). Previous work has documented elevated rates of the common deletion, a 4977-base-pair mtDNA deletion, in postmortem brain tissue of patients with bipolar disorder (35–39) and schizophrenia (40). Furthermore, increased rates of hydrogen peroxide–induced mtDNA lesions have been observed in peripheral blood mononuclear cells (PBMCs) of individuals with depression compared with healthy control participants (41). Deletions in mtDNA may compromise mitochondrial functioning, thereby contributing to disease processes. Quantifying mtDNA deletions may provide greater insight into mechanisms by which stress impacts critical cellular structures and processes and serves as an early warning marker of diseases. Furthermore, clarifying these mechanisms may allow for improved interventions for individuals with ELS and elevated risk for diseases. While existing science linking ELS to targeted interventions remains limited, a greater understanding of the physiological changes observed in ELS may provide new directions for future therapeutics.

Recent advancements in next-generation sequencing and bioinformatics tools now allow mtDNA deletions to be quantified at high resolution, with simultaneous detection of tens to thousands of unique mtDNA breakpoints in a human sample (42). The Splice-Break pipeline (40), updated for batch processing and additional deletion annotation (43), is one available method to quantify mtDNA deletions and can be utilized with several next-generation sequencing data types including libraries prepared from mitochondrial-enriched long-range polymerase chain reaction amplicons. Summary metrics of mtDNA deletions can be quantified and evaluated from this pipeline. Specifically, recent reports have focused on 1) the number of unique mtDNA deletions per 10,000 coverage, which is the number of different breakpoint species observed after normalization to mitochondrial chromosome sequencing depth, which negatively correlates with clonality, and 2) the cumulative deletion read percentage, which is the sum of all sequencing reads with a deletion after normalization to mitochondrial chromosome sequencing depth and positively correlates with cellular dysfunction (e.g., is increased in hallmark mtDNA deletion disorders) (42). The number of unique mtDNA deletion breakpoints per 10,000 coverage is increased in postmortem brains of individuals with schizophrenia and bipolar disorder (38), but no difference is observed for the cumulative deletion read percentage in these disorders (38,42).

To our knowledge, no previous studies have examined the associations of these summary mtDNA deletion metrics and childhood adversity. In the current study, we examined the number of unique mtDNA deletion breakpoints per 10,000 coverage and the cumulative deletion read percentage in PBMCs of medically healthy young adults with and without ELS and further assessed correlations with adult stressors, mental health characteristics, and demographic variables. We hypothesized that summary mtDNA deletion metrics might be altered in participants with ELS given previous studies that indicated altered mitochondrial structure and function following childhood adversity (22,25).

METHODS AND MATERIALS

Participants

This study included a subset of participants enrolled in the LIFE (Lifestyle Influences of Family Environment) study who provided blood samples. Participants (n = 181) were medically healthy young adults, ages 18 to 40 years, recruited via internet and community advertisements seeking healthy individuals raised in stable 2-parent households and individuals with childhood parental loss and other indicators of ELS. Participants were from Providence and nearby areas. Prospective participants were assessed for eligibility via phone screen. Prior to enrollment, participants were informed about the study, and voluntary written informed consent was obtained. The study was approved by the Butler Hospital Institutional Review Board.

Briefly, participants in the ELS group experienced moderate to severe childhood maltreatment before age 18 years, including physical, emotional, or sexual abuse and neglect. Most also had childhood parental loss, and some experienced interparental violence (see Measures). Participants in the control group were raised in 2-parent homes, with no parental separation or divorce, and had no major history of childhood maltreatment, parental loss, or psychiatric disorders. Participants with acute and chronic medical conditions, pregnancy, and the use of medications other than hormonal contraceptives were excluded. Participants were queried about recent exposures, and those with major acute stressors, illness, or sleep loss were rescheduled to a time with baseline exposures and behaviors. Positive drug screens were initially exclusionary, but to increase recruitment of eligible participants, this criterion was adjusted to allow inclusion of ELS participants who frequently used cannabis (3 or more times weekly or tested positive for cannabis, n = 15) and 1 control group participant who tested positive for cannabis. Because there is little work that has examined cannabis in mtDNA deletions, with the only experimental study to our knowledge showing potential protective effects of cannabis on mtDNA deletions in rodents (44), participants with cannabis use were included in analyses, and associations of mtDNA deletion summary

metrics and cannabis were controlled for in sensitivity analyses. Individuals with primary bipolar disorder, obsessive-compulsive disorder, and psychotic disorders were excluded. For a full description of the sampling methods, refer to Daniels *et al.* (45). A total of 220 participants enrolled in the study and met study inclusion/exclusion criteria. The final sample size of 181 included 108 participants with ELS and 73 participants without ELS after excluding participants who did not have usable blood samples or data for ELS group determination.

All LIFE study data were collected between October 2014 and March 2020. Individuals who met inclusion criteria participated in 2 visits for the current study, each approximately 1 week apart. Consent, medical history, anthropometrics, fasting blood draw, self-report measures, and the Structured Clinical Interview for DSM-5, Research Version 1.0.0 (46) were obtained during the first visit. The Childhood Experiences of Care and Abuse (CECA) interview was conducted during the second visit.

Measures

Demographics, Past Medical History, and Health Behaviors. Age, sex, gender, race, ethnicity, household income, and college graduate status were collected via participant self-report. A standardized interview was used to assess for medical problems, medications, and substance use.

Assessment of Early Adversity and Adult Stressors. Participants were included in the ELS and control groups based on information provided from the CECA, a validated semi-structured interview that assesses 8 objectively defined indices of adversity, including physical abuse, sexual abuse, parental neglect, psychological abuse, and antipathy, as well as childhood maternal or paternal loss and violence between parents or caregivers (47,48). A trained interviewer conducted the CECA, and scores were independently reviewed by another trained interviewer, with consensus scoring in unclear cases following group discussion. Participants in the ELS group experienced at least 1 form of maltreatment of moderate to marked severity before age 18. The Childhood Trauma Questionnaire (CTQ) 28-item version was also collected as a continuous self-report measure of childhood adversity. The CTQ evaluates physical, sexual, and emotional abuse and physical and emotional neglect on a 5-point Likert scale (49). For participants with missing CECA data (n = 6), telephone screens and CTQ data were used to determine the presence of maltreatment and parental loss.

To examine selective effects of childhood adversity, we collected measures of adult stressors with the Life Stress Questionnaire (LSQ), a self-report measure developed in our laboratory that assesses 47 common stressors and 2 free-response stressors during the past month, which has been described in Daniels *et al.* (45). We also used the Perceived Stress Scale (PSS), a psychometrically validated self-report questionnaire that assesses the degree to which people find their lives to be unpredictable, uncontrollable, or overcharged during the past month (50).

Psychiatric Disorders and Symptoms. Psychiatric diagnoses were assessed using the Structured Clinical Interview for DSM-5. Prior to the release of the DSM-5, a subset of participants (n = 34) was assessed using the Mini-International Neuropsychiatric Interview for the DSM-IV (MINI), which was adapted to include information for DSM-5 diagnoses (51). Anxiety was assessed using the Beck Anxiety Inventory, a validated 21-item self-report questionnaire of anxiety symptomatology experienced during the past week (52). Depression was assessed with the Inventory of Depressive Symptomatology Self-Report, a validated 30-item instrument that measures the severity of depressive symptoms during the past week (53).

Anthropometrics. Height and weight were measured using a Detecto scale/stadiometer, and body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Blood pressure was measured using a sphygmomanometer by a research nurse or a trained technician.

Blood Collection. Participants fasted from food and drink except water from 8:00 PM the evening before blood collection. Venipuncture was performed between 8:15 AM and 9:00 AM by a phlebotomist or a research nurse using standard practices. Blood was collected from the antecubital region in 4.5 mL sodium citrate tubes using an evacuated system with 23- to 21-gauge butterfly needles.

Isolation of PBMCs via Density Centrifugation (Ficoll-Paque Method) and Extraction. Human PBMCs were isolated from whole blood using a standard density gradient Ficoll-Paque approach (54). Isolated PBMC pellets were stored in an −80° C freezer for long-term storage. DNA was extracted using a Thermo Fisher Scientific KingFisher Flex System with the Omega Bio-Tek Mag-Bind Blood and Tissue DNA HDQ kit following manufacturer's instructions.

Cell-Type Analysis. Methylation of isolated PBMC DNA was assessed using the Illumina Infinium Human MethylationEPIC BeadChip (55), and the EpiDISH R package was used to infer the proportions of 7 cell types present (56).

Quantification of mtDNA Deletions. Utilizing the recently developed Splice-Break2 pipeline (https://github.com/brookehjelm/Splice-Break2) (42,43), mtDNA deletions were measured in PBMCs. The Splice-Break2 pipeline method is described in detail in the Supplement and in Hjelm *et al.* (42) and Omidsalar *et al.* (43).

Briefly, the mtDNA was enriched using a long-range polymerase chain reaction as previously described (38,42). Long-range polymerase chain reaction amplicons were used as input, and next-generation sequencing was performed using the 384 Seq-Well kit. Libraries were multiplex sequenced as 150-mer paired-end reads on a patterned flow cell using the Illumina NovaSeq6000 at the University of California Irvine Genomics High-Throughput Facility. FASTQ files were processed through the Splice-Break2 pipeline (42,43).

Two summary deletion metrics were evaluated to investigate the pooled effect of all mtDNA deletions, including 1) the number of unique mtDNA deletion breakpoints per 10,000 coverage, which is the number of distinct breakpoint species observed after normalization to sequencing depth, and 2) the

cumulative deletion read percentage, which is the sum of all sequencing reads with a deletion after normalization to sequencing depth.

Statistical Analyses

All analyses were performed using R (57). All participants (n = 181) were found to have summary mtDNA deletion metrics at levels similar to those observed in whole blood (42). The value distributions of the summary mtDNA deletions were skewed and thus were normalized using natural log transformation.

ELS group differences in demographic, stress, psychiatric, and biological variables were assessed using Student's t tests and χ^2 tests as appropriate. Due to limited representation across race, statistical analysis related to race was not performed. Pearson correlations were used to examine bivariate associations of the summary mtDNA deletion metrics, ELS, recent stress, and psychiatric and key demographic variables (38,42,58). Linear regression models were used to assess relationships between ELS and summary mtDNA deletion metrics. The average sequencing depth of two 250-bp fragments in the *RNR1* and *CYB* genes (43) was included as a covariate in Pearson correlations involving summary mtDNA deletion metrics. Consistent with previous work, sequencing depth was also included as a covariate in linear regression models (42), together with age and sex. Sensitivity analysis to distinguish ELS effects from contributions of psychiatric disorders, recent stress, or proportion of cell types in PBMC isolates and linear regression models examined relationships of unique mtDNA deletions per 10,000 coverage with psychiatric disorders, perceived stress (PSS), recent stress exposure (LSQ), ethnicity (Hispanic or not), cannabis usage, and cell type proportions. Participants with exceptions to study group inclusion/exclusion criteria, including cannabis use, were excluded in sensitivity analyses to assess their impact on the models.

RESULTS

Sample Characteristics

Demographic characteristics by group are presented in Table 1. Participants in the ELS group were more likely to identify as Hispanic and were less likely to have an annual household income above $50,000 or a college degree than those in the control group. BMI, but not waist-to-hip ratio, was greater for ELS participants. There were no group differences in hormonal contraceptive use, tobacco use, or blood pressure.

Table 1. Sample Characteristics

Demographics	Control Group, n = 73	ELS Group, n = 108	p Value
Age, Years	26.2 (5.5)	27.9 (5.7)	.052
Sex Assigned at Birth, Female	50 (68.5%)	75 (69.4%)	
Race			
American Indian/Alaska Native	0 (0.0%)	4 (3.7%)	
Asian	9 (12.3%)	4 (3.7%)	
Black or African American	5 (6.8%)	9 (8.3%)	
More than 1 race	2 (2.7%)	13 (12.0%)	
White	57 (78.1%)	69 (63.9%)	
Unknown	0 (0.0%)	9 (8.3%)	
Ethnicity, Hispanic	5 (6.8%)	26 (24.1%)	.005[a]
College Degree	53 (72.6%)	48 (44.4%)	<.001[b]
Household Income <$50,000	23 (34.3%)	66 (69.5%)	<.001[b]
Hormonal Contraceptive	25 (34.2%)	27 (25.0%)	.238
Tobacco Use, Current	7 (9.6%)	17 (15.7%)	.330
Cannabis Use, Any Use Reported	12 (16.4%)	43 (39.8%)	<.001[b]
Cannabis Use, Frequent User[c]	1 (1.37%)	15 (13.9%)	.004[a]
Anthropometrics			
Body mass index	25.91 (5.58)	28.72 (6.58)	.003[a]
Waist-to-hip ratio	0.84 (0.08)	0.85 (0.08)	.21
Systolic blood pressure, mm Hg	124.90 (13.86)	128.50 (13.39)	.09
Diastolic blood pressure, mm Hg	72.15 (8.32)	74.33 (8.07)	.08

Values are presented as n (%) or mean (SD). Statistical tests included χ^2 tests for sex assigned at birth, race, ethnicity, and college degree and 2-sided t tests for continuous variables.

ELS, early-life stress.

[a]Significant at the .01 level.

[b]Significant at the .001 level.

[c]Frequent user was defined as 3 or more times weekly or tested positive for cannabis.

Adversity and Psychiatric Characteristics

Participants in the ELS group had a high level of childhood adversity, with 74.1% having experienced 4 or more moderate to marked CECA adversity subtypes, 91.7% having experienced 3 or more moderate to marked adversity subtypes, and 98.1% having experienced 2 or more moderate to marked adversity subtypes. The mean number of adversity subtypes experienced in the ELS group was 4.9 of 8 possible subtypes. Individuals in the control group did not experience any CECA adversity subtypes. The frequencies of moderate to marked CECA adversity subtypes in the ELS group were as follows: 90.6% parental antipathy, 82.9% neglect, 70.2% physical abuse, 45.9% sexual abuse, 53.0% psychological abuse, 37.0% parental/caregiver violence, and 76.9% experiencing parental loss. The CTQ total score was significantly higher in the ELS group, with a mean of 64.9 compared with a mean of 27.2 among participants without ELS ($t_{114.5} = 19.91$, $p < .001$).

Recent adult stressors and psychiatric symptoms and disorders by group are presented in Table 2. Among participants with ELS, 59 (54.6%) had a current psychiatric diagnosis. Participants in the ELS group reported greater severity of symptoms of anxiety and depression, as measured by the Beck Anxiety Inventory and Inventory of Depressive Symptomatology Self-Report, respectively, and higher perceived stress on the PSS and more recent stressors on the LSQ.

Bivariate Correlations

Table 3 presents Pearson correlations (Pearson r values) and χ^2 values as appropriate for associations of summary mtDNA deletion metrics (unique mtDNA deletions per 10,000 coverage and cumulative mtDNA deletion read percentage), ELS

Table 2. Recent Stressors and Psychiatric Diagnoses and Symptomatology

	Control Group, n = 73	ELS Group, n = 108	p Value
Recent Stressors			
PSS Total	14.01 (5.89)	23.70 (8.68)	<.001[a]
LSQ Sum	1.46 (1.61)	3.82 (3.82)	<.001[a]
LSQ Weighted Score	3.86 (4.86)	11.75 (12.24)	<.001[a]
Psychiatric Disorders			
Current Disorders			
Any current diagnosis	0 (0.0%)	59 (54.6%)	
Depressive	0 (0.0%)	26 (24.2%)	
Trauma	0 (0.0%)	23 (21.3%)	
Anxiety	0 (0.0%)	36 (34.6%)	
Alcohol/substance	0 (0.0%)	13 (12.0%)	
Eating disorders	0 (0.0%)	6 (5.6%)	
Current Symptomatology, Self-Report			
IDS-SR total	6.04 (4.54)	18.18 (12.27)	<.001[a]
BAI total	1.88 (3.11)	7.79 (8.43)	<.001[a]

BAI, Beck Anxiety Inventory; ELS, early-life stress; IDS-SR, Inventory of Depressive Symptomatology Self-Report; LSQ, Life Stress Questionnaire; PSS, Perceived Stress Scale.

[a]Significant at the .001 level.

assessments, recent stress assessments, mental health conditions, and participant demographics or health metrics. The number of unique mtDNA deletion breakpoints per 10,000 coverage was positively associated with ELS group, the CECA adversity composite, CTQ total score, PSS perceived stress, and psychiatric disorder. There was a positive association between unique mtDNA deletion breakpoints per 10,000 coverage and LSQ total that did not reach significance. Cumulative mtDNA deletion read percentage was not associated with any metric of stress but was positively correlated with unique mtDNA deletion breakpoints per 10,000 coverage, which has been reported previously (40). Neither mtDNA deletion metric was associated with BMI, age, or sex. The ELS group also had significant and positive correlations with all stress measures as expected, as well as psychiatric disorder and BMI.

mtDNA Deletions

The number of unique mtDNA deletion breakpoints per 10,000 coverage ranged from 4.6 to 45.8, with a mean of 14.2 and a standard deviation of 7.1. The cumulative mtDNA deletion read percentage ranged from 0.09 to 3.54, with a mean of 0.42 and a standard deviation of 0.45. Summary mtDNA deletion metrics did not differ by sex.

Linear Regression Models Testing Associations of mtDNA Deletion Metrics and ELS. Linear regression models of unique mtDNA deletion breakpoints per 10,000 coverage and cumulative mtDNA deletion read percentage were tested with respect to ELS status and included sequencing depth, age, and sex as covariates (Figure 1). The number of unique mtDNA deletions per 10,000 coverage was significantly higher in the ELS group (β = 0.24; 95% CI, 0.12 to 0.36; p = .0001; adjusted R^2 = 0.22), and there was no significant group difference in cumulative mtDNA deletion read percentage (β = 0.20; 95% CI, −0.01 to 0.40; p = .06; adjusted R^2 = 0.03) (Figure 1).

Because the relationship between ELS group and unique mtDNA deletions per 10,000 coverage withstood adjustment for age, sex, and sequencing depth, we further investigated the effect of ELS by assessing the distinct contributions of ELS and current psychiatric disorders. When current psychiatric disorders was added to the linear regression model of unique mtDNA deletion breakpoints per 10,000 coverage with ELS

Table 3. Correlation Matrix Assessing Bivariate Associations Between Variables

	1	2	3	4	5	6	7	8	9	10
1) Unique mtDNA Deletion Breakpoints per 10,000 Coverage[a]										
2) Cumulative mtDNA Deletion Read Percentage[a]	0.514[b]									
3) ELS Group	0.281[b]	0.132								
4) CECA	0.235[c]	0.088	0.881[b]							
5) CTQ	0.230[c]	0.084	0.777[b]	0.855[b]						
6) Current Psychiatric Disorder	0.250[b]	0.152[d]	59.17[b]	0.517[b]	0.560[b]					
7) PSS	0.184[d]	0.100	0.530[b]	0.443[b]	0.518[b]	0.643[b]				
8) LSQ Total	0.172[d]	0.053	0.347[b]	0.248[c]	0.262[b]	0.353[b]	0.525[b]			
9) BMI	0.042	0.006	0.218[c]	0.199[c]	0.221[c]	0.240[c]	0.067	0.178[d]		
10) Age	0.021	−0.048	0.144	0.219[c]	0.175[d]	0.012	0.049	0.011	0.156[d]	
11) Sex	0.027	−0.073	0.018	0.093	0.164[d]	6.193[d]	0.107	−0.035	−0.024	0.079

Pearson correlation and χ^2 values are presented as appropriate. For sex, 0 = male, 1 = female.

BMI, body mass index; CECA, Childhood Experiences of Care and Abuse; CTQ, Childhood Trauma Questionnaire; ELS, early-life stress; LSQ, Life Stress Questionnaire; mtDNA, mitochondrial DNA; PSS, Perceived Stress Scale.

[a]Corrected for sequencing depth.

[b]Significant at the <.001 level (2-tailed).

[c]Significant at the <.01 level (2-tailed).

[d]Significant at the <.05 level (2-tailed).

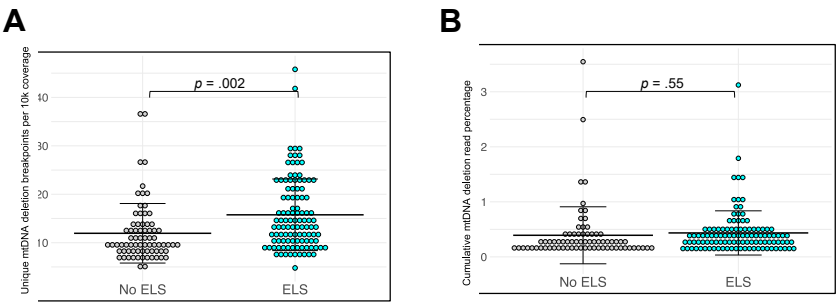

Figure 1. Summary mtDNA deletion metrics in peripheral blood mononuclear cells (raw data) by ELS group. **(A)** Unique mtDNA deletion breakpoints per 10,000 coverage by ELS group. **(B)** Cumulative mtDNA deletion read percentage by ELS group. Statistical results reported from linear regression models of summary mtDNA deletion metrics by ELS group and includes age, sex, and sequencing depth as covariates. ELS, early-life stress; mtDNA, mitochondrial DNA.

group and covariates age, sex, and sequencing depth, ELS group remained a significant predictor (β = 0.17; 95% CI, 0.03–0.32; p = .022; adjusted R^2 = 0.22), and psychiatric disorders were not associated with unique mtDNA deletion breakpoints (p = .15). Sequencing depth also remained significant (β = 0.34; $p < .001$), although age and sex were not (p = .87 and p = .97). Furthermore, among only the participants with ELS, the presence of a current psychiatric disorder did not significantly predict greater unique mtDNA deletion species (β = 0.09; 95% CI, −0.07 to 0.26; p = .26).

To distinguish the effects of ELS from perceived stress and recent stressors, we included perceived stress (via the PSS) and recent stressors (via the LSQ) in individual linear regression models predicting unique mtDNA deletion breakpoints per 10,000 coverage with ELS group and the covariates as previously. In each model, ELS remained significant (β = 0.20; 95% CI, 0.06 to 0.34; p = .006; adjusted R^2 = 0.21; β = 0.22; 95% CI, 0.09 to 0.35; p = .001; adjusted R^2 = 0.23), and neither perceived stress (β = 0.002, p = .61) nor recent stressors (β = 0.01, p = .28) was significant.

To further confirm that the association of ELS status and unique mtDNA deletion breakpoints per 10,000 coverage was not due to differences in ethnicity or cannabis use, these covariates were also tested in our linear models. When adjusting for cannabis use, the ELS group remained significant for mtDNA deletions (p = .006). Neither ethnicity (p = .51) nor cannabis use (p = .92) was significant.

Sensitivity Analyses

Sensitivity analyses were conducted to rule out effects related to deviations from the group inclusion/exclusion criteria described above (i.e., ELS group participants with exclusionary psychiatric disorders). For these sensitivity tests, each subgroup was systematically excluded from the model, and in each case, the predictive relationship of adversity and unique mtDNA deletion breakpoint species per 10,000 coverage remained substantially unchanged.

Additional models (not shown) were conducted to rule out effects of related variables that were significantly different between ELS groups and could potentially account for some of the observed variance, including college degree and household income. In individual models that included ELS grouping variable and the covariate, none of the covariates were found to be significant predictors of mtDNA deletions, and therefore they were not considered further. A sensitivity analysis was performed adjusting for the proportion of each of 7 cell types using the EpiDISH analysis, and the results remained unchanged (data not shown).

DISCUSSION

To our knowledge, this is the first study to examine the relationship of childhood adversity with a high-resolution analysis of unique mtDNA deletion breakpoints per 10,000 coverage and cumulative deletion read percentage in PBMCs. The results presented here indicate that ELS was associated with greater rates of unique species of mtDNA deletion breakpoints, and this significant association remained after testing for factors of age, sex, sequencing depth, psychiatric disorders, recent stress, and cell-type proportions. These findings add to the growing body of literature demonstrating the impact of childhood adversity on physiological changes in mitochondria and provide further evidence indicating that stress-associated mitochondrial mechanisms may contribute to downstream health outcomes (21,59,60).

The greater rates of unique mtDNA deletion breakpoints per 10,000 coverage observed in healthy individuals with early adversity highlights the potential impact of childhood adverse experiences on mitochondria. Given the broad range of critical functions of mitochondria, increased alterations of the mitochondrial genome observed in ELS may contribute substantially to aging and health risk. As with other work that has demonstrated alterations of mtDNA in adversity, including effects of adversity on mtDNA copy number (22,23,25,26) and functional effects on mitochondrial respiration (61–64), these findings add to the growing evidence of mitochondrial stress sensitivity in early life (65). The implications of increased rates of unique mtDNA deletion breakpoints are not clear; however, a recent study revealed that chronic stress is associated with hypermetabolic mitochondria, a characteristic highly associated with unstable mtDNA (66). While the results presented here do not provide a clear mechanistic explanation for the relationship between ELS and impacts on mitochondrial health, hypothetical models suggest that ELS may impact mtDNA via increased demand, oxidative stress, and inflammatory processes (18,19,21). Furthermore, increased mtDNA deletions are associated with disease processes that have previously been linked to ELS, such as insulin resistance, atherosclerosis, and neurodegenerative disorders, suggesting a mechanistic role for stress-associated mtDNA deletions in these processes (33,34,67). Quantification of mtDNA deletions

may complement self-report data in ELS to provide insights about biological changes that occur in stress and trauma, and considering mtDNA deletions along with other mitochondrial biomarkers impacted in stress may point to mechanisms by which ELS impacts downstream health risk. These findings highlight that future work, particularly longitudinal studies, are needed to examine mechanistic relationships between ELS, mitochondrial health, and health outcomes.

The science linking targeted therapies to mtDNA deletions is limited at this time (33); however, mtDNA deletions could offer a pathway toward more tailored interventions. Rates of mtDNA deletions may allow for earlier detection of stress-related diseases, stratification of patients into risk categories, specific recommendations for interventions and lifestyle changes, and potentially direction for targeted pharmacological approaches that impact mitochondrial integrity. While the relationship of this work to downstream therapies is hypothetical, this finding may represent a step toward improved understanding of how ELS affects physiology and new directions for future therapies.

Furthermore, this study found that while psychiatric disorders and recent stress were associated with unique mtDNA deletion breakpoints, these did not account for the ELS associations with unique mtDNA deletions. The effects of psychiatric disorders and recent stress were no longer significant when included in models with ELS group, suggesting that these effects may be due to ELS. In previous studies, psychiatric disorders were found to be associated with mtDNA deletions (38,42), although these samples often also included participants with bipolar disorder and schizophrenia, which were excluded from the current sample, and results were from postmortem brain tissue and not PBMCs. The impact of mtDNA deletions and mutations on psychiatric function remains unclear; however, it should be noted that populations with mitochondrial disorders marked by mtDNA deletions have greater rates of psychiatric disorders (68), and mtDNA damage has been shown to be associated with increased risk of psychiatric disorders (69).

We did not observe a difference in the cumulative deletion read percentage between participants with and without ELS. Similarly, previous studies did not observe significant differences in this metric in postmortem brains of participants with psychiatric disorders, although it was significantly increased with age in the brain but not in blood (42). Interestingly, unique mtDNA deletion breakpoints per 10,000 coverage has been observed to be increased in some, but not all, brain regions in individuals with psychiatric disorders but was not observed to increase with age in a cohort of mixed mental health conditions (38,42). The levels that we observed are consistent with our previous measures of whole blood and are many folds less than what is observed in brain or muscle (42,43).

One important question that we cannot answer is whether the increased number of unique mtDNA deletion breakpoints in PBMCs of participants with ELS is also observable in other biofluids or tissues. If the effects of childhood adversity are systemic, similar trends may be observable in saliva, muscle, or brain tissue. Likewise, it would be important to evaluate these mtDNA deletion measurements in repeated measures, such as blood collections taken over time from the same participants, to determine the stability and lifetime trends of this initial observation.

The findings presented here should be considered in the retrospective and observational study design context. Confounding effects of genetic or prenatal variables cannot be excluded, and assessment of ELS, although rigorous and well validated (48), may be confounded by recall bias (70). Furthermore, given the cross-sectional structure of this study, we cannot infer a causal relationship between early adversity and mtDNA deletions. Nevertheless, the effects of potentially confounding variables (such as age, sex, sequencing depth, cell-type proportion, and recent stress) did not account for the findings presented here. Future work should examine stress and mtDNA deletions prospectively and longitudinally to clarify the specificity of effects and to assess the dynamics of these relationships throughout the life course and as they relate to health outcomes.

It is also important to interpret these findings in the context of the current literature. Deletions represent a mutation to the genome and thus may be harmful to the functioning of mitochondria (67) and in turn impact a person's health risk. Substantially more work is required to understand the mechanisms and risk associated with mtDNA deletions to identify actionable interventions in the future. Furthermore, interventions that are aimed at reducing mitochondrial damage and promoting repair mechanisms may help mitigate harmful effects; however, interventions that directly reverse mtDNA deletions are not currently available.

Limitations to the demographic representation within the sample should be noted. There was limited representation of diverse gender identities, and future studies should examine the effects of childhood adversity in gender-diverse samples (71). Additionally, participants predominantly identified as White, with more significant Hispanic ethnicity among participants with childhood adversity. Future work should include participants with greater diversity in genetic ancestry and cultural identity and examine factors that contribute to childhood adversity, including structural factors such as systemic racism (72). Critically, when interpreting differences related to structural factors and discrimination, race and ethnicity are unlikely to be causal factors (73,74), and including more samples with genetic diversity can help uncover relationships with cis-effects on mtDNA deletions (75). Future samples should also have comparable cannabis use across groups. Additional tests of comorbidities, environmental exposures, and diseases associated with ELS will also be necessary to further understand the downstream consequences of these mitochondrial alterations.

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ART and MPV conceived of the study. TED and ART were responsible for the study design. AS collected and processed data. Sample sequencing was conducted by BLR and supervised by MPV. AAO processed the data through Splice-Break2 and generated the figures. BEH and WWL-dlA conducted the data analyses. TED drafted the manuscript. BEH, ART, and ES contributed to original draft. All authors reviewed and edited the manuscript. All authors discussed and approved the manuscript.

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ARTICLE INFORMATION

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