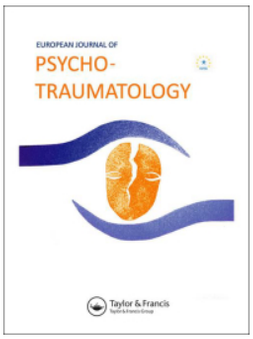

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BASIC RESEARCH ARTICLE

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No association between war-related trauma or PTSD symptom severity and epigenome-wide DNA methylation in Burundian refugees

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ABSTRACT

Background: War-related trauma is associated with varying posttraumatic stress disorder (PTSD) prevalence rates in refugees. In PTSD development, differential DNA methylation (DNAm) levels associated with trauma exposure might be involved in risk versus resilience processes. Studies investigating DNAm profiles related to trauma exposure and PTSD among refugees remain sparse.
Objective: The present epigenome-wide association study investigated associations between war-related trauma, PTSD, and altered DNAm patterns in Burundian refugee families with 110 children and their 207 female and male caregivers.
Method: War-related trauma load and PTSD symptom severity were assessed in structured clinical interviews with standardised instruments. Epigenome-wide DNAm levels were quantified from buccal epithelia using the Illumina EPIC beadchip.
Results: Controlling for biological confounders, no significant epigenome-wide DNAm alterations associated with trauma exposure or PTSD were identified in children or caregivers (*FDRs* > .05). Co-methylated positions derived as modules from weighted gene correlation network analyses were not significantly associated with either war-related trauma experience in children or caregivers or with PTSD.
Conclusions: These results do not provide evidence for altered DNAm patterns associated with exposure to war-related trauma or PTSD.

No hay Asociación entre el trauma relacionado con la Guerra o gravedad de los síntomas de TEPT y la metilación del ADN de todo el epigenoma en refugiados de Burundi

Antecedentes: El trauma relacionado con la guerra se asocia con diferentes tasas de prevalencia de trastorno de Estrés postraumático (TEPT) en refugiados. En el desarrollo del TEPT, los niveles diferenciales de metilación del ADN (ADNm) con exposición al trauma podrían estar involucrados en los procesos de riesgo versus resiliencia. Los estudios que investigan los perfiles de ADNm relacionados con la exposición al trauma y TEPT entre refugiados siguen siendo escasos.
Objetivo: El presente estudio de asociación de todo el epigenoma investigó las asociaciones entre el trauma relacionado con la guerra, TEPT y patrones de ADNm alterados en familias de refugiados de Burundi con 110 niños y sus 207 cuidadores femeninos y masculinos.
Método: La carga traumática relacionada con la guerra y la gravedad de los síntomas del TEPT se evaluaron en entrevistas clínicas estructuradas con instrumentos estandarizados. Los niveles de ADNm de todo el epigenoma fueron cuantificados a partir del epitelio bucal utilizando el chip de cuentas Illlumina EPIC.
Resultados: Al controlar los factores de confusión biológicos, no se identificaron alteraciones significativas en el ADNm en todo el epigenoma asociadas con la exposición al trauma o TEPT en niños o cuidadores (*FDRs* > .05). Las posiciones co-metiladas derivadas como módulos de los análisis de redes de correlación de genes ponderados no se asociaron significativamente ni con experiencias traumáticas relacionadas con la guerra en niños o cuidadores ni con TEPT.
Conclusiones: Estos resultados no proporcionan evidencia de patrones de ADNm alterados asociados con la exposición a traumas relacionados con la guerra o TEPT.

布隆迪难民的战争相关创伤或 PTSD 症状严重程度与全表观基因组DNA 甲基化之间没有关联

背景：难民中战争相关创伤与不同的创伤后应激障碍 (PTSD) 流行率有关。 在 PTSD 发展中，与创伤暴露相关的不同 DNA 甲基化 (DNAm) 水平可能涉及风险或恢复过程。考查难民中创伤暴露和PTSD相关的 DNAm 剖面的研究仍然很少。

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KEYWORDS
Burundian refugee families; war trauma; PTSD; DNA methylation; EWAS; WGCNA

PALABRAS CLAVE
Familias de refugiados de Burundi; trauma de guerra; TEPT; metilación del ADN; EWAS; WGCNA

关键词
布隆迪难民家庭; 战争创伤; PTSD; DNA 甲基化; EWAS; WGCNA

HIGHLIGHTS

- The study examines an understudied population in epigenome-wide association studies.
- Burundian refugees' war-trauma, PTSD, and DNA methylation were studied.
- Epigenome-wide DNA methylation was not significantly associated with war-trauma or PTSD in the conflict-affected sample.

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目的：本全表观基因组关联研究考查了布隆迪难民家庭（110 名儿童及其 207名女性和男性照顾者）中战争相关创伤、PTSD 和改变的 DNAm 模式之间的关联。
方法：在使用标准化工具的结构化临床访谈中对战争相关创伤负荷和 PTSD 症状严重程度进行评估。使用 Illumina EPIC 珠芯片从口腔上皮细胞中量化全表观基因组DNAm水平。
结果：控制生物混杂因素后，在儿童或看护者中未发现与创伤暴露或 PTSD 相关的显著全表观基因组 DNAm 改变 (*FDR* > .05)。从加权基因相关网络分析识别为模块的共甲基化位置与儿童或看护者的战争相关创伤经历或 PTSD 均无显著相关性。
结论：这些结果并未提供与战争相关创伤暴露或 PTSD 相关的 DNAm 模式改变的证据。

1. Background

It is estimated that more than 50% of refugees who fled armed conflicts are affected by mental health problems including posttraumatic stress disorder (PTSD) (Attanayake et al., 2009; Fazel et al., 2005; Neuner et al., 2004; Scharpf, Kaltenbach, et al., 2021; Shawyer et al., 2017; Steel et al., 2002). Recent meta-analyses showed that PTSD has one of the highest prevalence rates in refugees: between 4% and 63% in adult refugees and 7% and 40% in refugee minors across studies (Blackmore, Boyle, et al., 2020; Blackmore, Gray, et al., 2020; Mesa-Vieira et al., 2022).

Varying PTSD prevalence rates in refugees can be partly attributed to methodological differences and cultural heterogeneity of the study samples. However, studies in war and crisis regions have repeatedly shown a strong association between trauma load and PTSD development, even after adjusting for methodological factors (Neuner et al., 2004; Steel et al., 2009; Wilker et al., 2015). Yet, trauma load does not seem to explain the varying prevalence rates sufficiently. There is a considerable portion of individuals with trauma experience who do not develop PTSD even after multiple or severe traumatic exposures (Bonanno, 2004; Hoge et al., 2004; Kessler et al., 1995; Steel et al., 2009), suggesting that individual vulnerability and resilience to trauma are key factors in PTSD development (Ford et al., 2015).

PTSD development is thought to result from interactions between environmental and personal risk factors (Ford et al., 2015). Increasing evidence suggests that one mechanism for gene by environment interactions that differentiates risk versus resilience involves epigenetic processes (Yehuda & Bierer, 2009; Yehuda & LeDoux, 2007). The most studied epigenetic mark in the field of mental health is DNA methylation (DNAm), a chemical modification of DNA at cytosine-guanine dinucleotides (CpGs) which can alter gene regulation (Novik et al., 2002). It has been suggested that the epigenome can accommodate environmental influences in the form of long-lasting DNAm modifications which alter the central nervous system's structures and functioning (Aristizabal et al., 2020). Such alterations can originate from exposures to traumatic events during sensitive periods of development but have also been described as a result of adult exposures to trauma (Mill & Heijmans, 2013).

To identify differential DNAm patterns associated with PTSD risk, two strategies have been followed (Morrison et al., 2019). Candidate gene studies identify genes of interest based on presumed biological pathways involved in PTSD development to address potential mechanistic links between epigenetic alterations and PTSD risk. As PTSD is highly linked to stress responses (Dunlop & Wong, 2019) and inflammatory reactions (Kim et al., 2020), differential DNAm of genes involved in these regulations has been mainly investigated with the candidate gene approach. Investigations of genes involved in stress and inflammatory regulations in individuals with and without PTSD including survivors of the Rwandan genocide (Perroud et al., 2014; Rudahindwa et al., 2020; Vukojevic et al., 2014) have shown differential DNAm in promoter regions of genes encoding for exon 1F of the glucocorticoid receptor (GR) gene (*NR3C1*) (Labonte et al., 2014; Vukojevic et al., 2014), FK506 binding protein 51 (*FKBP5*), a regulator of GR sensitivity (Klengel & Binder, 2015; Zannas et al., 2016), glucocorticoid-induced leucine zipper (*GILZ*, *TSC22D3*) (Lebow et al., 2019), a GR responsive transcript with anti-inflammatory properties and a potential indicator of glucocorticoid pathway sensitivity (Cheng et al., 2014; Thiagarajah et al., 2014), stress responsive pituitary adenylate cyclase-activating polypeptide (*ADCYAP1R1*) (Ressler et al., 2011), and spindle and kinetochore associated protein 2 (*SKA2*), which plays a role in glucocorticoid receptor transactivation (Boks et al., 2016; Sadeh et al., 2016). Increasingly, these gene targeted studies are being criticised for lack of replicability, risk of false positive results, and for potentially missing unexpected biological pathways involved in disorder etiology due to its narrow focus on genes related to presumed biological pathways involved in PTSD development (Morrison et al., 2019).

Epigenome-wide association studies (EWAS), in contrast, offer an unbiased approach identifying novel candidate gene pathways potentially implicated in PTSD risk (Morrison et al., 2019). Multiple studies so far have examined DNAm alterations in PTSD using EWAS. Following earlier PTSD-EWAS with

small sample sizes (Hammamieh et al., 2017; Mehta et al., 2017; Smith et al., 2011; Uddin et al., 2010), more recent large-scale EWAS with civilians and military cohorts report various differentially DNAm changes associated with trauma and PTSD (Katrinli et al., 2022; Logue et al., 2020; Montalvo-Ortiz et al., 2022; Smith et al., 2020; Snijders et al., 2020). Still, EWAS among African individuals who have experienced war- and conflict-related trauma are rare (Carleial et al., 2021; Musanabaganwa et al., 2022). Broadly, the existing EWAS findings in PTSD generally align with the current understanding that two major biological processes – the stress response and immune system – are involved in PTSD etiology (Mehta et al., 2020).

Taken together, the aforementioned evidence indicates a conjunction of exposure to war- and conflict-related trauma with an increased PTSD risk, and epigenetic processes have been proposed as an involved mechanism (Morrison et al., 2019). However, evidence linking trauma exposure and/or PTSD to epigenetic alterations in conflict-affected African populations remains sparse. To address this research gap, the present study investigated whether epigenome-wide DNAm patterns are associated with war-related trauma load and PTSD symptom severity in a cohort of Burundian refugee families living in refugee camps. This sample was particularly suited for our research question because the sample was homogeneous in their traumatic experiences, ethnic and genetic background, current living situation, and, although the participants showed a very high level of trauma exposure in form of war-related violence, they varied widely in their PTSD symptom levels (cf., Scharpf et al., 2019).

2. Methods

The study methods are briefly summarised here and provided in detail in Scharpf et al. (2019) and Scharpf, Mkinga, et al. (2021).

2.1. Study setting and participants

The study was conducted with refugee families from Burundi living in refugee camps in Tanzania. After several outbreaks of extreme political and ethnic violence and a civil war (1993–2005) (Irankunda et al., 2017), Burundi faced political conflict in 2015. More than 300,000 Burundians fled to neighbouring countries to escape violence and atrocities committed by ruling party members towards perceived opponents, including abductions, extrajudicial killings, and torture (World Report, 2017).

Data from 230 family triads comprising the oldest child in primary school age (7–15 years), and the female and male primary caregiver ($N = 690$) was collected in three refugee camps (Nyarugusu, Mtendeli, & Nduta) in the Kigoma region of Western Tanzania between January and June 2018. Within the camps, tents or small makeshift houses provided temporary shelter for refugees. Each camp was divided into zones and two zones were randomly selected. Due to the absence of reliable census data, a sampling direction was randomly determined by spinning a pen at the zone centre in each of the selected zones. In the chosen direction, eligible family triads in every sixth household were invited for study participation.

2.2. Ethics approval and consent to participate

The study was approved by the Ethics Commission of the University of Zurich, Switzerland (2017.10.2), Ruhr-University Bochum, Germany (639), and National Institute for Medical Research, Dar es Salaam, Tanzania (NIMR/HQ/R.8a/Vol.IX/2632). The study was conducted in accordance with the ethical principles for medical research involving human subjects as defined by the Declaration of Helsinki. The participants received detailed explanation of the study purpose, procedure, associated risks, their right of participation withdrawal at any time, and data confidentiality. Before data assessment, caregivers provided written informed consent for their own and their child's participation by name or fingerprint. Children below the age of 11 gave oral assent, while children aged 11 and above additionally gave written informed consent.

2.3. Study procedures

Data collection was conducted simultaneously with the child and both caregivers in discrete settings within the camps. After the individual interviews, each family member provided a buccal swab.

2.3.1. Structured clinical interview

Structured clinical interviews were conducted by three Tanzanian psychological researchers and three trained study assistants from the refugee community in Kirundi (Burundian's mother language) or Swahili (lingua franca in all 3 camps) translated to Kirundi. The instruments have been applied successfully in previous studies with children in primary school in Tanzania (Hecker et al., 2016) and Congolese refugees in Uganda (Ainamani et al., 2017) and the applicability of the instruments was evaluated in a pilot assessment of eight families. Sociodemographic information on participant's sex and age was assessed with purpose-built questions (inspired by Ainamani et al., 2017). War-related trauma load was measured through dichotomously coded items adapted from the Violence, War, and Abductee Exposure Scale (Ertl et al., 2010) (possible range [pr]: 0–22 [children], 0–26

[caregivers]). The caregivers reported on four additional items related to perpetrating war-related violence introducing the differing possible ranges of sum scores between children and caregivers. PTSD symptom severity was assessed by the sum score of 31 items from the University of California at Los Angeles PTSD Reaction Index for DSM-5 (Pynoos & Steinberg, 2013) for the children and for the caregivers by 20 items from the PTSD Check List for DSM-5 (Weathers et al., 2013) (5-point Likert scales [0–4]). Both instruments measure the frequency of specific symptomatic reactions to traumatic events in the past month (pr: 0–124 [children], 0–80 [caregivers]). Caregivers' substance use was measured by the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) (Humeniuk et al., 2010) using eight items. The total substance involvement score was indicated by the sum score of all items for all assessed drug classes (alcohol, tobacco, cannabis, & maximum 7 other substances specified by participant; pr: 0–440). Since not reported in Scharpf et al. (2019), the internal consistency of the ASSIST was good (Cronbach's alpha = .85) in our sample. Strong correlations with the Mini International Neuropsychiatric Interview (.76) (Sheehan et al., 1998) and Addiction Severity Index (.84) (McLellan et al., 1992) support the ASSIST's validity.

2.3.2. Collection and analysis of buccal epithelium samples

A buccal epithelium sample was collected from each participant using an Isohelix SK-1S Buccal Swab (Cell Projects Ltd, Kent, UK) following manufacturer's instructions. The swab, removed from the numerically-labelled tube, was rubbed against inside the cheek. Then, a silica gel Isohelix Dri-Capsule (Cell Projects Ltd, Kent, UK) was applied to the swab head and placed into the tube and the tube was sealed. The capsule is ideal for field sample collection, since it enables long term stability of DNA samples within the tube at room temperature and reduces DNA cross contamination risk. DNA samples were available from 201 family triads ($N = 603$).

2.4. DNA isolation and genome-wide methylation assessment

Genomic DNA was isolated using standard salting out procedure from 456 archived buccal epithelium samples in the Genetic Psychology laboratory, Ruhr-University Bochum, Germany. Due to logistics problems during field research and transportation, 147 buccal samples could not be processed. Additionally, due to low DNA amount, 24 samples could not be quantified and were discarded. Bisulfite conversion and assessment of genome-wide DNAm levels at more than 850,000 CpGs using the Infinium HumanMethylationEPIC BeadChip array (Illumina, San Diego, CA, USA) were performed for 432 DNA samples following the manufacturer's protocol at the Life&Brain Core Facility (Bonn University, Germany).

2.5. Data analysis

Data analyses were performed after study registration (https://osf.io/tx4ue) with R (R Core Team, 2021) using RStudio 4.2.2. For all inferential analyses, the Benjamini-Hochberg approach was applied to the empirical p-values adjusting for multiple testing with a p-value cutoff of a False Discovery Rate (*FDR*; so-called q-value) < .05. Downstream analyses that required significant results from preceding analyses (i.e. differentially methylated regions, pathway enrichment, & mediations) were not conducted. Post-hoc sensitivity analyses accounting for potential technical batch effects by SentrixID were conducted.

2.5.1. DNA methylation data preprocessing

Raw Infinium EPIC data were preprocessed for intensity summarisation and calculation of DNAm ratio (beta-values) using the RnBeads-pipeline (Assenov et al., 2014; Müller et al., 2019). During preprocessing, the quality control step removed 79 samples due to low bisulfite conversion efficiency, the first filtering step eliminated cross-reactive, SNP (single nucleotide polymorphism)-enriched probes, and based on the RnBeads´ Greedycut algorithm with default settings removed low-quality probes and samples (36). The normalisation step normalised the DNAm data by the dasen-method (Pidsley et al., 2013) implemented in RnBeads, and the second filtering step removed context-specific CpGs, CpGs on sex chromosomes, and CpGs with missing beta-values. These steps lead to a final set of 715,211 CpGs for each sample in a total sample size of 317.

2.5.2. Assessment of cell type heterogeneity

To account for cell type heterogeneity in the DNAm profiles, a reference-based approach was used to estimate cell type compositions from the beta-values of the assessed DNAm data using the *HEpiDISH*-function with the CIBERSORT-algorithm from the R-package EpiDISH (Newman et al., 2015; Teschendorff & Zheng, 2017; Zheng et al., 2018). This approach has been successfully applied previously to DNAm profiles from epithelia cells in adults and children (Camerota et al., 2021; Odintsova et al., 2019; Odintsova et al., 2021; Wong et al., 2022).

2.5.3. EWAS

A series of sex-combined EWAS using the limma R-package (Ritchie et al., 2015) was conducted separately for children and caregivers. Multiple regression models accounting for outlier-robust empirical Bayes

moderation on the M-values (logit-transformed beta-values) of each retrieved CpG as criterion and war-related trauma load or PTSD symptom severity as continuous predictor were used to identify differentially methylated positions (DMPs). For each predictor, a model was implemented. In all models, a set of a-priori theory-based selected additive covariates of DNAm levels (age [years], sex, technical batch [assay plate], & estimated cell type proportions [in terms of model parsimony 3 non-collinear cell types for children (epithelia, CD4 T, & eosinophils) and for caregivers 4 (epithelia, natural killer, CD4 T, & eosinophils)]) were included simultaneously. In all caregiver's models, substance use known to be associated with DNAm (Cecil et al., 2015) was included as an additional additive confounder of DNAm. For each regression model, outlier CpGs indicating hemimethylated sites were excluded to avoid false-positive associations (Mansell et al., 2019).

2.5.4. WGCNA

DNAm data were clustered by employing weighted gene correlation network analysis (WGCNA) (Langfelder & Horvath, 2008) to the top 50% of the preprocessed CpGs (each participant: 357,606 CpGs), ranked by median absolute deviation (MAD). WGCNA offers a systems-level view of DNAm data as CpGs are clustered based on DNAm level correlations indicating similarity in their methylation profiles. Using the R-package WGCNA (Langfelder & Horvath, 2008), weighted co-methylation modules representing discrete networks of co-methylated CpGs were constructed using scale-free correlation-based hierarchical clusters of CpGs. An unsigned network with outlier-robust biweight midcorrelation, blockwise module detection of pre-clustered blocks, and soft-threshold (estimated by scale free topology analysis: $ß_{children} = 6$, $ß_{caregivers} = 3$) were chosen. Based on the topological overlap approach and average linkage hierarchical clustering, MAD-filtered CpGs with high interconnectedness were assigned into modules. Each module was labelled by a colour for module distinction and summarised by the EigenCpG (first principal component of variance). This procedure identified 33 modules for the children and 37 modules for the caregivers. Lastly, the modules' EigenCpGs were linearly regressed onto war-related trauma load or PTSD symptom severity including the same confounders of the EWAS.

3. Results

In all analyses, 317 participants were included: 110 children (49% female) and 207 caregivers (53% female). Participants' sociodemographic data are presented in Table 1 and the descriptive results of the variables assessed in the interview in Table 2.

Table 1. Sociodemographic characteristics in children and caregivers.

	Children ($n = 110$)	Caregivers ($n = 207$)
Age in years, *M* (*SD*, range)	12.2 (2.1, 7–15)	37.4 (10.3, 19–74)
Education, *n*		
No schooling	10	57
Class 1–3 (primary)	58	37
Class 4–6	41	74
Some secondary school years	1	28
Completed secondary school	–	11
Country of origin, *n*		
Burundi	74	194
Tanzania	36	10
Democratic Republic of Congo	0	2
Other	0	1
Household size, *M* (*SD*, range)	7.2 (1.8, 3–12)	7.3 (1.9, 3–13)
Household income per month (USD), *n*		
No income	–	69
Up to 20	–	115
More than 20	–	23

Note: *n* = conditional sample size, *M* = mean, *SD* = standard deviation, USD = US-Dollar, – = not applicable.

Table 2. Prevalence rates of mental health problems in the child and caregiver samples.

	Children ($n = 110$)		Caregivers ($n = 207$)	
	Female ($n = 54$)	Male ($n = 56$)	Female ($n = 108$)	Male ($n = 99$)
Number of war-related traumatic event types, *M* (*SD*)	3.2 (3.0)	4.0 (4.0)	12.2 (4.5)	15.5 (4.1)
PTSD diagnosis, *n*	2	4	39	27
PTSD symptom severity, *M* (*SD*)	15.2 (11.8)	20.3 (16.4)	40.5 (19.2)	34.1 (16.3)
Substance use, *M* (*SD*)	–	–	7.7 (11.9)	17.4 (17.8)

Note: The caregivers' measures significantly differed between the sexes (*ps* < .05), the children's did not (*ps* > .05). *n* = conditional sample size, *M* = mean, *SD* = standard deviation, PTSD = post-traumatic stress disorder, – = not applicable.

In the EWAS, analyses on the WGCNA-modules and the post-hoc sensitivity analyses, neither war-related trauma load nor PTSD symptom severity were significantly associated with the epigenome-wide DNAm levels of the retrieved CpGs nor the module's EigenCpGs from WGCNA, neither for the children nor for the caregivers (*FDRs* > .05; Figures 1 and 2; Supplementary Tables S1–S3).

4. Discussion

Our EWAS and WGCNA investigating potential associations between war-related trauma exposure or PTSD symptom severity and altered DNAm profiles in refugee families did not identify any significant results controlling for biological and technical confounders and multiple testing. These results are in line with a previously reported PTSD-EWAS with the Illumina 450k chip. Kuan et al. (2017) reported *FDR*-corrected null findings in an EWAS with 473 World Trade Center responders with PTSD diagnosis. However, other EWAS did report significant

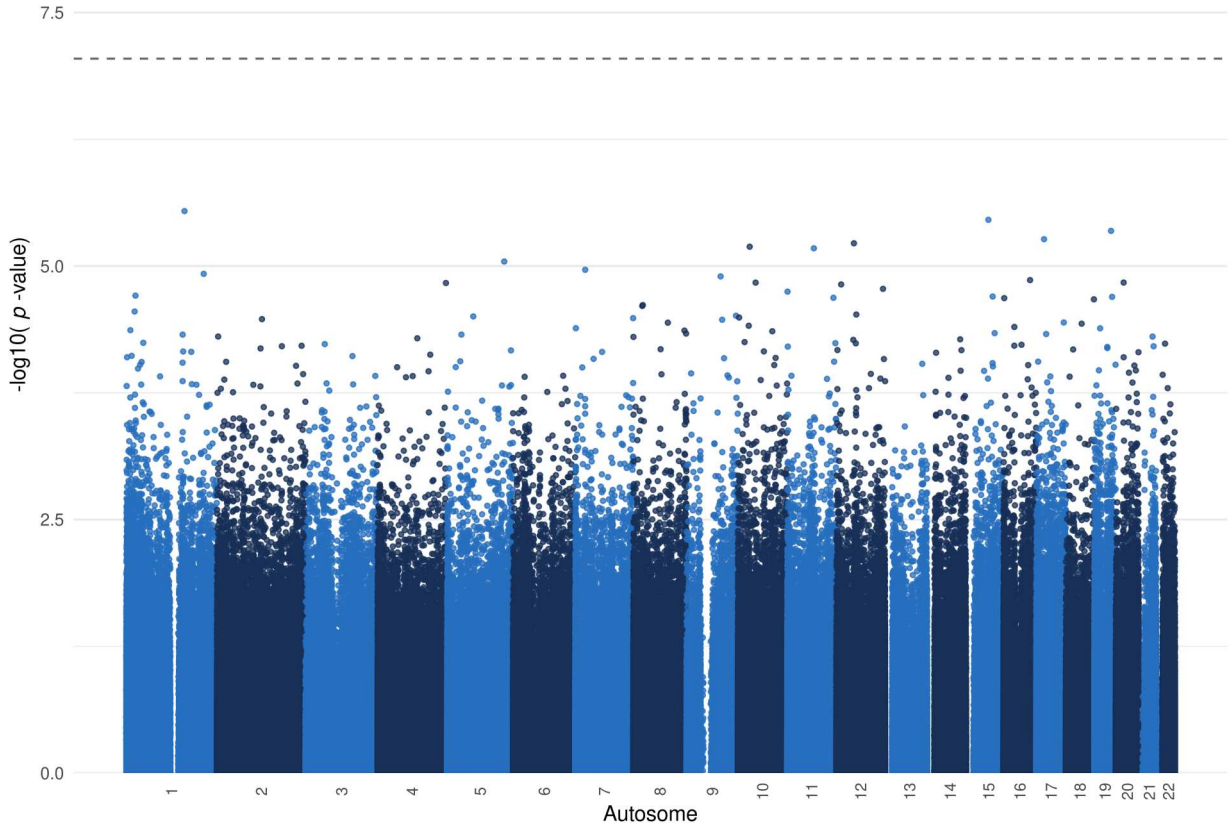

Figure 1. Stacked manhattan plot of retrieved CpGs from EWAS for children. For the sake of illustration, −log10 transformed *p*-value of each CpG analysed in the two children's EWAS including either the predictor of war-related trauma load or PTSD symptom severity are plotted in order of its location on the autosome. CpGs on the sex chromosomes were not analysed. The dashed line indicates the array-wide nominal threshold of $-\log10(9 \times 10^{-8})$. There were no significant DMPs associated with any children's predictor.

alterations of epigenome-wide DNAm profiles associated with PTSD in traumatised participants: Recent EWAS on large-scale cohorts reported multiple epigenome-wide DNA methylation changes associated with PTSD using the state-of-the-art assays (Katrinli et al., 2022; Logue et al., 2020; Montalvo-Ortiz et al., 2022; Smith et al., 2020). These well-powered EWAS that followed the preprocessing pipeline for best practice investigated civilian and military non-African cohorts with and without PTSD diagnosis after exposure to different trauma types. Here, we investigated a conflict-affected African population. Since epigenetic profiles are population specific with little concordance in DNAm profiles between populations, generalisation of results regarding DNAm from a specific population to other populations is restricted (Fraser et al., 2012). At the current state of research, trauma and PTSD-EWAS among conflict-affected African individuals are underrepresented. There is one EWAS with 84 female former child soldiers from the Eastern Democratic Republic of Congo using the EPIC assay, which identified one DMP significantly associated with PTSD diagnosis (Carleial et al., 2021). However, this finding was not replicated by Smith et al. (2011) in a sample of 53 female former child soldiers from Northern Uganda.

Several limitations of our study might account for our null-findings. First, we assessed the epigenome-wide DNAm levels from peripheral buccal epithelia cells, and any potential influence of war-related trauma on the epigenome might not be reflected in buccal epithelium. In general, it is still unclear to what extent DNAm patterns can be used as a proxy for the brain, the tissue of interest in PTSD. Second, the buccal swabs were taken from Burundian refugees living in refugee camps where oral hygiene was poor (Calderón-Villarreal et al., 2022). Potential bacterial contamination of the human DNA in the buccal samples was addressed by only including CpGs with high quality based on quality control during preprocessing. Third, with the available sample size, we were not adequately powered to detect small effects, so that unidentified false-negative results cannot be ruled out in our study. Of note, no post-hoc power analysis was run since observed power is generally based on collected data and thus is non-informative about the true power (Lakens, 2013). Forth, due to the power issue, we were not able to address interaction effects between the predictor of interests and the participants' sex. Since there is general strong evidence that trauma load as well as PTSD symptom severity differ between sexes (Hodes & Epperson, 2019;

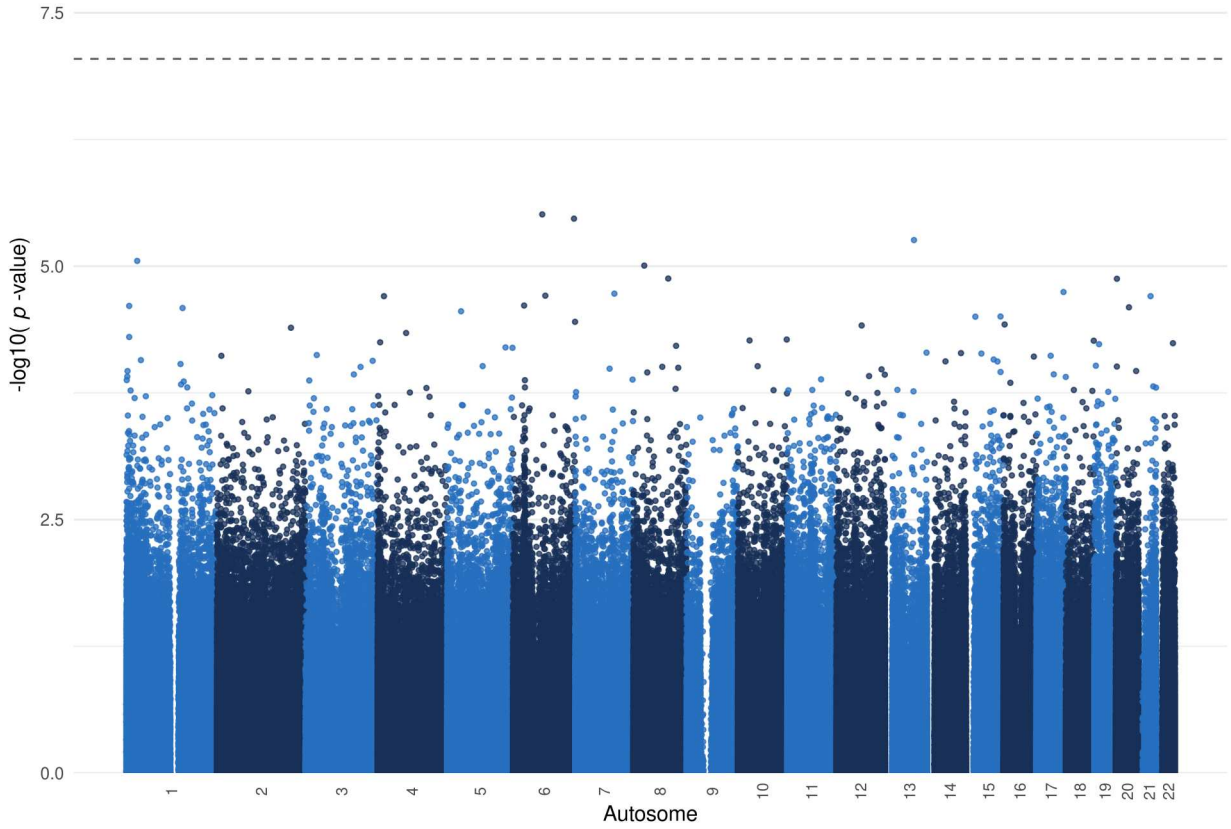

Figure 2. Stacked manhattan plot of retrieved CpGs from EWAS for caregivers. For the sake of illustration, −log10 transformed *p*-value of each CpG analysed in the two caregivers' EWAS including either the predictor of war-related trauma load or PTSD symptom severity are plotted in order of its location on the autosome. CpGs on the sex chromosomes were not analysed. The dashed line indicates the array-wide nominal threshold of $-\log10(9 \times 10^{-8})$. There were no significant DMPs associated with any caregivers' predictor.

Nievergelt et al., 2019), which also holds true for our caregiver samples, future studies with larger sample size should account for such interaction effects to be able to identify potential significant effects on DNAm profiles.

A strength of our study is that we regressed the epigenome-wide DNAm on the actual trauma load as well as PTSD symptom severity in contrast to previously reported EWAS using a case–control approach with participants with and without PTSD diagnosis as indirect indicator of trauma exposure. In order to understand the dynamics in DNAm profiles, linearly regressing the outcome on continuous predictors generally gives detailed insight into the form of association that is generally not addressable using group comparison. Beyond associative dynamics, future studies would benefit from a longitudinal approach to account for temporal dynamics in DNAm signatures established and changed by pre-clinical, clinical, and post-clinical conditions of trauma exposure and PTSD (Morrison et al., 2019). Another strength of our study is that we assessed children as well as adults being the child's primary caregivers. Future EWAS with larger sample size of children and caregivers could give insight into multilevel effects between family members. Our study appears to be the first study specifically addressing epigenetic signatures of trauma and PTSD in refugee children and their caregivers from Africa. Thus, with this study, we contribute to the generally understudied population of African individuals since other ethnic populations appear to be overrepresented in PTSD-EWAS.

To conclude, understanding psychological and biological underpinnings of behavioural vulnerability and resilience to traumatic stress is a public health priority, as it could facilitate the development of preventive strategies and therapeutic interventions for trauma-related disorders, including PTSD (Nemeroff et al., 2006; Taki & de Melo-Martin, 2021). Future large-scale EWAS with underrepresented conflict-affected African populations are needed for a better understanding on effects of war-related trauma and PTSD on DNAm in African individuals.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Health and safety

We confirm that all mandatory laboratory health and safety procedures have been complied with in the course of conducting the research reported in our paper.

Consent for publication

Each participant gave written informed consent that the results may be published for scientific purposes provided that the participant's identity is not revealed and cannot be reconstructed.

Data availability statement

DNAm and phenotypic data are not publicly available due to de-anonymization risk but available from the corresponding author on reasonable request.

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References

Ainamani, H., Elbert, T., Olema, D., & Hecker, T. (2017). PTSD symptom severity relates to cognitive and psycho-social dysfunctioning – a study with Congolese refugees in Uganda. *European Journal of Psychotraumatology*, *8*(1), 1283086. doi:10.1080/20008198.2017.1283086

Aristizabal, M. J., Anreiter, I., Halldorsdottir, T., Odgers, C. L., McDade, T. W., Goldenberg, A., Mostafavi, S., Kobor, M. S., Binder, E. B., Sokolowski, M. B., & O'Donnell, K. J. (2020). Biological embedding of experience: A primer on epigenetics. *Proceedings of the National Academy of Sciences*, *117*(38), 23261–9. doi:10.1073/pnas.1820838116

Assenov, Y., Müller, F., Lutsik, P., Walter, J., Lengauer, T., & Bock, C. (2014). Comprehensive analysis of DNA methylation data with RnBeads. *Nature Methods*, *11*(11), 1138–1140. doi:10.1038/nmeth.3115

Attanayake, V., McKay, R., Joffres, M., Singh, S., Burkle, F., & Mills, E. (2009). Prevalence of mental disorders among children exposed to war: A systematic review of 7,920 children. *Medicine, Conflict and Survival*, *25*(1), 4–19. doi:10.1080/13623690802568913

Blackmore, R., Boyle, J. A., Fazel, M., Ranasinha, S., Gray, K. M., Fitzgerald, G., Misso, M., & Gibson-Helm, M. (2020). The prevalence of mental illness in refugees and asylum seekers: A systematic review and meta-analysis. *PLOS Medicine*, *17*(9), e1003337. doi:10.1371/journal.pmed.1003337

Blackmore, R., Gray, K. M., Boyle, J. A., Fazel, M., Ranasinha, S., Fitzgerald, G., Misso, M., & Gibson-Helm, M. (2020). Systematic review and meta-analysis: The prevalence of mental illness in child and adolescent refugees and asylum seekers. *Journal of the American Academy of Child & Adolescent Psychiatry*, *59*(6), 705–714. doi:10.1016/j.jaac.2019.11.011

Boks, M. P., Rutten, B. P. F., Geuze, E., Houtepen, L. C., Vermetten, E., Kaminsky, Z., & Vinkers, C. H. (2016). SKA2 methylation is involved in cortisol stress reactivity and predicts the development of post-traumatic stress disorder (PTSD) after military deployment. *Neuropsychopharmacology*, *41*(5), 1350–1356. doi:10.1038/npp.2015.286

Bonanno, G. A. (2004). Loss, trauma, and human resilience: Have we underestimated the human capacity to thrive after extremely aversive events? *American Psychologist*, *59*(1), 20–28. doi:10.1037/0003-066X.59.1.20

Calderón-Villarreal, A., Schweitzer, R., & Kayser, G. (2022). Social and geographic inequalities in water, sanitation and hygiene access in 21 refugee camps and settlements in Bangladesh, Kenya, Uganda, South Sudan, and Zimbabwe. *International Journal for Equity in Health*, *21*(1), 27. doi:10.1186/s12939-022-01626-3

Camerota, M., Graw, S., Everson, T. M., McGowan, E. C., Hofheimer, J. A., O'Shea, T. M., Carter, B. S., Helderman, J. B., Check, J., Neal, C. R., Pastyrnak, S. L., Smith, L. M., Dansereau, L. M., DellaGrotta, S. A., Marsit, C. J., & Lester, B. M. (2021). Prenatal risk factors and neonatal DNA methylation in very preterm infants. *Clinical Epigenetics*, *13*(1), 171. doi:10.1186/s13148-021-01164-9

Carleial, S., Nätt, D., Unternährer, E., Elbert, T., Robjant, K., Wilker, S., Vukojevic, V., Kolassa, I.-T., Zeller, A. C., & Koebach, A. (2021). DNA methylation changes following narrative exposure therapy in a randomized controlled trial with female former child soldiers. *Scientific Reports*, *11*(1), 18493. doi:10.1038/s41598-021-98067-9

Cecil, C. A. M., Walton, E., & Viding, E. (2015). DNA methylation, substance use and addiction: A systematic review of recent animal and human research from a developmental perspective. *Current Addiction Reports*, *2*(4), 331–346. doi:10.1007/s40429-015-0072-9

Cheng, Q., Morand, E., & Yang, Y. H. (2014). Development of novel treatment strategies for inflammatory diseases—similarities and divergence between glucocorticoids and GILZ. *Frontiers in Pharmacology*, *5*, 169. doi:10.3389/fphar.2014.00169

Dunlop, B. W., & Wong, A. (2019). The hypothalamic-pituitary-adrenal axis in PTSD: Pathophysiology and treatment interventions. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *89*, 361–379. doi:10.1016/j.pnpbp.2018.10.010

Ertl, V., Pfeiffer, A., Saile, R., Schauer, E., Elbert, T., & Neuner, F. (2010). Validation of a mental health assessment in an African conflict population. *Psychological Assessment*, *22*(2), 318–324. doi:10.1037/a0018810

Fazel, M., Wheeler, J., & Danesh, J. (2005). Prevalence of serious mental disorder in 7000 refugees resettled in western countries: A systematic review. *Lancet*, *365*(9467), 1309–1314. doi:10.1016/S0140-6736(05)61027-6

Ford, J. D., Grasso, D. J., Elhai, J. D., & Courtois, C. A. (2015). Etiology of PTSD. In *Posttraumatic stress disorder* (2nd ed., pp. 81–132). Elsevier. doi:10.1016/B978-0-12-801288-8.00003-0

Fraser, H. B., Lam, L. L., Neumann, S. M., & Kobor, M. S. (2012). Population-specificity of human DNA methylation. *Genome Biology*, *13*(2), R8. doi:10.1186/gb-2012-13-2-r8

Hammamieh, R., Chakraborty, N., Gautam, A., Muhie, S., Yang, R., Donohue, D., Kumar, R., Daigle, B. J., Zhang, Y., Amara, D. A., Miller, S.-A., Srinivasan, S., Flory, J., Yehuda, R., Petzold, L., Wolkowitz, O. M., Mellon, S. H., Hood, L., Doyle, F. J., … Jett, M. (2017). Whole-genome DNA methylation status associated with clinical PTSD measures of OIF/OEF veterans. *Translational Psychiatry*, *7*(7), e1169. doi:10.1038/tp.2017.129

Hecker, T., Hermenau, K., Salmen, C., Teicher, M., & Elbert, T. (2016). Harsh discipline relates to internalizing problems and cognitive functioning: Findings from a cross-sectional study with school children in Tanzania. *BMC Psychiatry*, *16*(1), 118. doi:10.1186/s12888-016-0828-3

Hodes, G. E., & Epperson, C. N. (2019). Sex differences in vulnerability and resilience to stress across the life span. *Biological Psychiatry*, *86*(6), 421–432. doi:10.1016/j.biopsych.2019.04.028

Hoge, C. W., Castro, C. A., Messer, S. C., McGurk, D., Cotting, D. I., & Koffman, R. L. (2004). Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. *New England Journal of Medicine*, *351*(1), 13–22. doi:10.1056/NEJMoa040603

Humeniuk, R. E., Henry-Edwards, S., Ali, R. L., Poznyak, V., & Monteiro, M. (2010). *The alcohol, smoking and substance involvement screening test (ASSIST). Manual for use in primary care*. World Health Organization.

Irankunda, P., Heatherington, L., & Fitts, J. (2017). Local terms and understandings of mental health problems in Burundi. *Transcultural Psychiatry*, *54*(1), 66–85. doi:10.1177/1363461516689004

Katrinli, S., Maihofer, A. X., Wani, A. H., Pfeiffer, J. R., Ketema, E., Ratanatharathorn, A., Baker, D. G., Boks, M. P., Geuze, E., Kessler, R. C., Risbrough, V. B., Rutten, B. P. F., Stein, M. B., Ursano, R. J., Vermetten, E., Logue, M. W., Nievergelt, C. M., Smith, A. K., & Uddin, M. (2022). Epigenome-wide meta-analysis of PTSD symptom severity in three military cohorts implicates DNA methylation changes in genes involved in immune system and oxidative stress. *Molecular Psychiatry*, *27*(3), 1720–1728. doi:10.1038/s41380-021-01398-2

Kessler, R. C., Sonnega, A., Hughes, M., Bromet, E., & Nelson, C. B. (1995). Posttraumatic stress disorder in the national comorbidity survey. *Archives of General Psychiatry*, *52*(12), 1048–1060. doi:10.1001/archpsyc.1995.03950240066012

Kim, T. D., Lee, S., & Yoon, S. (2020). Inflammation in posttraumatic stress disorder (PTSD): A review of potential correlates of PTSD with a neurological perspective. *Antioxidants (Basel)*, *9*(2), 107. doi:10.3390/antiox9020107

Klengel, T., & Binder, E. B. (2015). FKBP5 Allele-specific epigenetic modification in gene by environment interaction. *Neuropsychopharmacology*, *40*(1), 244–246. doi:10.1038/npp.2014.208

Kuan, P.-F., Waszczuk, M. A., Kotov, R., Marsit, C. J., Guffanti, G., Gonzalez, A., Yang, X., Koenen, K., Bromet, E., & Luft, B. J. (2017). An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Translational Psychiatry*, *7*(6), e1158. doi:10.1038/tp.2017.130

Labonte, B., Azoulay, N., Yerko, V., Turecki, G., & Brunet, A. (2014). Epigenetic modulation of glucocorticoid receptors in posttraumatic stress disorder. *Translational Psychiatry*, *4*(3), e368. doi:10.1038/tp.2014.3

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*, 863. doi:10.3389/fpsyg.2013.00863

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1), 559. doi:10.1186/1471-2105-9-559

Lebow, M. A., Schroeder, M., Tsoory, M., Holzman-Karniel, D., Mehta, D., Ben-Dor, S., Gil, S., Bradley, B., Smith, A. K., Jovanovic, T., Ressler, K. J., Binder, E. B., & Chen, A. (2019). Glucocorticoid-induced leucine zipper "quantifies" stressors and increases male susceptibility to PTSD. *Translational Psychiatry*, *9*(1), 178. doi:10.1038/s41398-019-0509-3

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., … Verfaellie, M. (2020). An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clinical Epigenetics*, *12*(1), 46. doi:10.1186/s13148-020-0820-0

Mansell, G., Gorrie-Stone, T. J., Bao, Y., Kumari, M., Schalkwyk, L. S., Mill, J., & Hannon, E. (2019). Guidance for DNA methylation studies: Statistical insights from the Illumina EPIC array. *BMC Genomics*, *20*(1), 366. doi:10.1186/s12864-019-5761-7

McLellan, A. T., Kushner, H., Metzger, D., Peters, R., Smith, I., Grissom, G., Pettinati, H., & Argeriou, M. (1992). The fifth edition of the addiction severity index. *Journal of Substance Abuse Treatment*, *9*(3), 199–213. doi:10.1016/0740-5472(92)90062-S

Mehta, D., Bruenig, D., Carrillo-Roa, T., Lawford, B., Harvey, W., Morris, C. P., Smith, A. K., Binder, E. B., Young, R. M., & Voisey, J. (2017). Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. *Acta Psychiatrica Scandinavica*, *136*(5), 493–505. doi:10.1111/acps.12778

Mehta, D., Miller, O., Bruenig, D., David, G., & Shakespeare-Finch, J. (2020). A systematic review of DNA methylation and gene expression studies in posttraumatic stress disorder, posttraumatic growth, and resilience. *Journal of Traumatic Stress*, *33*(2), 171–180. doi:10.1002/jts.22472

Mesa-Vieira, C., Haas, A. D., Buitrago-Garcia, D., Roa-Diaz, Z. M., Minder, B., Gamba, M., Salvador, D., Gomez, D., Lewis, M., Gonzalez-Jaramillo, W. C., Pahud de Mortanges, A., Buttia, C., Muka, T., Trujillo, N., & Franco, O. H. (2022). Mental health of migrants with pre-migration exposure to armed conflict: A systematic review and meta-analysis. *The Lancet Public Health*, *7*(5), e469–e481. doi:10.1016/S2468-2667(22)00061-5

Mill, J., & Heijmans, B. T. (2013). From promises to practical strategies in epigenetic epidemiology. *Nature Reviews Genetics*, *14*(8), 585–594. doi:10.1038/nrg3405

Montalvo-Ortiz, J. L., Gelernter, J., Cheng, Z., Girgenti, M. J., Xu, K., Zhang, X., Gopalan, S., Zhou, H., Duman, R. S., Southwick, S. M., Krystal, J. H., Friedman, M. J., Duman, R. S., Girgenti, M. J., Krystal, J. H., Montalvo-Ortiz, J. L., & Pietrzak, R. H. (2022). Epigenome-wide association study of posttraumatic stress disorder identifies novel loci in U.S. military veterans. *Translational Psychiatry*, *12*(1), 65. doi:10.1038/s41398-022-01822-3

Morrison, F. G., Miller, M. W., Logue, M. W., Assef, M., & Wolf, E. J. (2019). DNA methylation correlates of PTSD: Recent findings and technical challenges. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *90*, 223–234. doi:10.1016/j.pnpbp.2018.11.011

Müller, F., Scherer, M., Assenov, Y., Lutsik, P., Walter, J., Lengauer, T., & Bock, C. (2019). RnBeads 2.0: Comprehensive analysis of DNA methylation data. *Genome Biology*, *20*(1), 55. doi:10.1186/s13059-019-1664-9

Musanabaganwa, C., Wani, A. H., Donglasan, J., Fatumo, S., Jansen, S., Mutabaruka, J., Rutembesa, E., Uwineza, A., Hermans, E. J., Roozendaal, B., Wildman, D. E., Mutesa, L., & Uddin, M. (2022). Leukocyte methylomic imprints of exposure to the genocide against the Tutsi in Rwanda: A pilot epigenome-wide analysis. *Epigenomics*, *14*(1), 11–25. doi:10.2217/epi-2021-0310

Nemeroff, C. B., Bremner, D., Foa, E. B., Mayberg, H. S., North, C. S., & Stein, M. B. (2006). Posttraumatic stress disorder: A state-of-the-science review. *Journal of Psychiatric Research*, *40*(1), 1–21. doi:10.1016/j.jpsychires.2005.07.005

Neuner, F., Schauer, M., Klaschik, C., Karunakara, U., & Elbert, T. (2004). A comparison of narrative exposure therapy, supportive counseling, and psychoeducation for treating posttraumatic stress disorder in an African refugee settlement. *Journal of Consulting and Clinical Psychology*, *72*(4), 579–587. doi:10.1037/0022-006X.72.4.579

Newman, A. M., Liu, C. L., Green, M. R., Gentles, A. J., Feng, W., Xu, Y., Hoang, C. D., Diehn, M., & Alizadeh, A. A. (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nature Methods*, *12*(5), 453–457. doi:10.1038/nmeth.3337

Nievergelt, C. M., Maihofer, A. X., Klengel, T., Atkinson, E. G., Chen, C. Y., Choi, K. W., Coleman, J. R. I., Dalvie, S., Duncan, L. E., Gelernter, J., Levey, D. F., Logue, M. W., Polimanti, R., Provost, A. C., Ratanatharathorn, A., Stein, M. B., Torres, K., Aiello, A. E., Almli, L. M., … Koenen, K. C. (2019). International meta-analysis of PTSD genome-wide association studies identifies sex- and ancestry-specific genetic risk loci. *Nature Communications*, *10*(1), 4558. doi:10.1038/s41467-019-12576-w

Novik, K. L., Nimmrich, I., Genc, B., Maier, S., Piepenbrock, C., Olek, A., & Beck, S. (2002). Epigenomics: Genome-wide study of methylation phenomena. *Current Issues in Molecular Biology*, *4*(4), 111–128. doi:10.21775/cimb.004.111

Odintsova, V., Sudermann, M., Hagenbeek, F., Caramaschi, D., Hottenga, J. J., Pool, R., Dolan, C., Ligthart, L., van Beijsterveldt, C., Willemsen, G., de Geus, E., Beck, J., Ehli, E., Cuellar-Partida, G., Evans, D., Medland, S., Relton, C., Boomsma, D., & van Dongen, J. (2021). Epigenome-wide association study of left-handedness for different tissues and ages. *Research Square* [Preprint], doi:10.21203/rs.3.rs-375556/v1

Odintsova, V. V., Hagenbeek, F. A., Suderman, M., Caramaschi, D., van Beijsterveldt, C. E. M., Kallsen, N. A., Ehli, E. A., Davies, G. E., Sukhikh, G. T., Fanos, V., Relton, C., Bartels, M., Boomsma, D. I., van Dongen, J. (2019). DNA methylation signatures of breastfeeding in buccal cells collected in mid-childhood. *Nutrients*, *11*(11), 2804. doi:10.3390/nu11112804

Perroud, N., Rutembesa, E., Paoloni-Giacobino, A., Mutabaruka, J., Mutesa, L., Stenz, L., Malafosse, A., & Karege, F. (2014). The Tutsi genocide and transgenerational transmission of maternal stress: Epigenetics and biology of the HPA axis. *The World Journal of Biological Psychiatry*, *15*(4), 334–345. doi:10.3109/15622975.2013.866693

Pidsley, R., Wong, C. C. Y., Volta, M., Lunnon, K., Mill, J., & Schalkwyk, L. C. (2013). A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics*, *14*(1), 293. doi:10.1186/1471-2164-14-293

Pynoos, R. S., & Steinberg, A. M. (2013). *UCLA PTSD reaction index for children/adolescents – DSM-5*. University of California.

R Core Team. (2021). *R: A language and environment for statistical computing* [Internet]. R Foundation for Statistical Computing. https://www.R-project.org/.

Ressler, K. J., Mercer, K. B., Bradley, B., Jovanovic, T., Mahan, A., Kerley, K., Norrholm, S. D., Kilaru, V., Smith, A. K., Myers, A. J., Ramirez, M., Engel, A., Hammack, S. E., Toufexis, D., Brass, K. M., Binder, E. B., & May, V. (2011). Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature*, *470*(7335), 492–497. doi:10.1038/nature09856

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7), e47. doi:10.1093/nar/gkv007

Rudahindwa, S., Mutesa, L., Rutembesa, E., Mutabaruka, J., Qu, A., Wildman, D. E., Jansen, S., & Uddin, M. (2020). Transgenerational effects of the genocide against the Tutsi in Rwanda: A post-traumatic stress disorder symptom domain analysis [version 2; peer review: 1 approved, 1 approved with reservations]. *AAS Open Research*, *1*, 10. doi:10.12688/aasopenres.12848.1

Sadeh, N., Wolf, E. J., Logue, M. W., Hayes, J. P., Stone, A., Griffin, L. M., Schichman, S. A., & Miller, M. W. (2016). Epigenetic variation at Ska2 predicts suicide phenotypes and internalizing psychopathology. *Depression and Anxiety*, *33*(4), 308–315. doi:10.1002/da.22480

Scharpf, F., Kaltenbach, E., Nickerson, A., & Hecker, T. (2021). A systematic review of socio-ecological factors contributing to risk and protection of the mental health of refugee children and adolescents. *Clinical Psychology Review*, *83*, 101930. doi:10.1016/j.cpr.2020.101930

Scharpf, F., Kyaruzi, E., Landolt, M. A., & Hecker, T. (2019). Prevalence and co-existence of morbidity of posttraumatic stress and functional impairment among Burundian refugee children and their parents. *European Journal of Psychotraumatology*, *10*(1), 1676005. doi:10.1080/20008198.2019.1676005

Scharpf, F., Mkinga, G., Neuner, F., Machumu, M., & Hecker, T. (2021). Fuel to the fire: The escalating interplay of attachment and maltreatment in the transgenerational transmission of psychopathology in families living in refugee camps. *Development and Psychopathology*, *33*(4), 1308–1321. doi:10.1017/S0954579420000516

Shawyer, F., Enticott, J. C., Block, A. A., Cheng, I. H., & Meadows, G. N. (2017). The mental health status of refugees and asylum seekers attending a refugee health clinic including comparisons with a matched sample of Australian-born residents. *BMC Psychiatry*, *17*(1), 76. doi:10.1186/s12888-017-1239-9

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The mini-international neuropsychiatric interview (M.I.N.I): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(20), 22–33.

Smith, A. K., Conneely, K. N., Kilaru, V., Mercer, K. B., Weiss, T. E., Bradley, B., Tang, Y., Gillespie, C. F., Cubells, J. F., & Ressler, K. J. (2011). Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *156* (6), 700–708. doi:10.1002/ajmg.b.31212

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., … Nievergelt, C. M. (2020). Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies methylation changes in AHRR. *Nature Communications*, *11*(1), 5965. doi:10.1038/s41467-020-19615-x

Snijders, C., Maihofer, A. X., Ratanatharathorn, A., Baker, D. G., Boks, M. P., Geuze, E., Jain, S., Kessler, R. C., Pishva, E., Risbrough, V. B., Stein, M. B., Ursano, R. J., Vermetten, E., Vinkers, C. H., Smith, A. K., Uddin, M., Rutten, B. P. F., & Nievergelt, C. M. (2020). Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clinical Epigenetics*, *12*(1), 11. doi:10.1186/s13148-019-0798-7

Steel, Z., Chey, T., Silove, D., Marnane, C., Bryant, R. A., & Van Ommeren, M. (2009). Association of torture and other potentially traumatic events with mental health outcomes among populations exposed to mass conflict and displacement: A systematic review and meta-analysis. *JAMA*, *302*(5), 537–549. doi:10.1001/jama.2009.1132

Steel, Z., Silove, D., Phan, T., & Bauman, A. (2002). Long-term effect of psychological trauma on the mental health of Vietnamese refugees resettled in Australia: A population-based study. *Lancet*, *360*(9339), 1056–1062. doi:10.1016/S0140-6736(02)11142-1

Taki, F., & de Melo-Martin, I. (2021). Conducting epigenetics research with refugees and asylum seekers: Attending to the ethical challenges. *Clinical Epigenetics*, *13*(1), 105. doi:10.1186/s13148-021-01092-8

Teschendorff, A. E., & Zheng, S. C. (2017). Cell-type deconvolution in epigenome-wide association studies: A review and recommendations. *Epigenomics*, *9*(5), 757–768. doi:10.2217/epi-2016-0153

Thiagarajah, A. S., Eades, L. E., Thomas, P. R., Guymer, E. K., Morand, E. F., Clarke, D. M., & Leech, M. (2014). GILZ: Glitzing up our understanding of the glucocorticoid receptor in psychopathology. *Brain Research*, *1574*, 60–69. doi:10.1016/j.brainres.2014.06.008

Uddin, M., Aiello, A. E., Wildman, D. E., Koenen, K. C., Pawelec, G., de los Santos, R., Goldmann, E., & Galea, S. (2010). Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proceedings of the National Academy of Sciences*, *107* (20), 9470–9475. doi:10.1073/pnas.0910794107

Vukojevic, V., Kolassa, I.-T., Fastenrath, M., Gschwind, L., Spalek, K., Milnik, A., Heck, A., Vogler, C., Wilker, S., Demougin, P., Peter, F., Atucha, E., Stetak, A., Roozendaal, B., Elbert, T., Papassotiropoulos, A., & de Quervain, D. J.-F. (2014). Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. *Journal of Neuroscience*, *34*(31), 10274–10284. doi:10.1523/JNEUROSCI.1526-14.2014

Weathers, F. W., Litz, B. T., Keane, T. M., Palmieri, P. A., Marx, B. P., & Schnurr, P. P. (2013). The PTSD Checklist for DSM-5 (PCL-5). National Center for PTSD.

Wilker, S., Pfeiffer, A., Kolassa, S., Koslowski, D., Elbert, T., & Kolassa, I. T. (2015). How to quantify exposure to traumatic stress? Reliability and predictive validity of measures for cumulative trauma exposure in a post-conflict population. *European Journal of Psychotraumatology*, *6*(1), 28306. doi:10.3402/ejpt.v6.28306

Wong, Y. T., Tayeb, M. A., Stone, T. C., Lovat, L. B., Teschendorff, A. E., Iwasiow, R., & Craig, J. M. (2022). A comparison of epithelial cell content of oral samples estimated using cytology and DNA methylation. *Epigenetics*, *17*(3), 327–334. doi:10.1080/15592294.2021.1950977

World Report. (2017). Burundi | Human Rights Watch. 2017.

Yehuda, R., & Bierer, L. M. (2009). The relevance of epigenetics to PTSD: Implications for the DSM-V. *Journal of Traumatic Stress*, *22*(5), 427–434. doi:10.1002/jts.20448

Yehuda, R., & LeDoux, J. (2007). Response variation following trauma: A translational neuroscience approach to understanding PTSD. *Neuron*, *56*(1), 19–32. doi:10.1016/j.neuron.2007.09.006

Zannas, A. S., Wiechmann, T., Gassen, N. C., & Binder, E. B. (2016). Gene–stress–epigenetic regulation of FKBP5: Clinical and translational implications. *Neuropsychopharmacology*, *41*(1), 261–274. doi:10.1038/npp.2015.235

Zheng, S. C., Breeze, C. E., Beck, S., & Teschendorff, A. E. (2018). Identification of differentially methylated cell types in epigenome-wide association studies. *Nature Methods*, *15*(12), 1059–1066. doi:10.1038/s41592-018-0213-x