# Associations between war exposure and DNA methylation in Syrian refugee children and adolescents

## Authors

Demelza Smeeth, PhD[1,2], Simone Ecker, PhD [3], Olga Chervova, PhD[3], Fiona McEwen, PhD[1,4], Elie Karam, MD[5], Stephan Beck, PhD[3], Michael Pluess, PhD[1,2]

## Affiliations

[1] Department of Biological and Experimental Psychology, School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK

[2] School of Psychology, University of Surrey, Guildford, UK

[3] UCL Cancer Institute, University College London, London, UK

[4] Department of War Studies, King's College London, London, UK

[5] Department of Psychiatry and Clinical Psychology, Balamand University, St Georges Hospital University Medical Center, Institute for Development, Research, Advocacy and Applied Care (IDRAAC), Lebanon

Word count: 2973

Revised: 15th September 2024

Correspondence to: Professor Michael Pluess, 05AC05 Lewis Carrol Building, School of Psychology, Faculty of Health & Medical Sciences, University of Surrey, Guildford, GU2 7XH, UK; Email: m.pluess@surrey.ac.uk; Telephone number: +44 (0)1483 68 9762

## Key words

**Question:** What are the salivary DNA methylation (DNAm) differences associated with war exposure in Syrian refugee children and adolescents?

**Findings:** In this Original Investigation of 1507 Syrian refugee children and adolescents, war exposure was associated with differential methylation at various sites and regions, but not at sites previously linked to childhood trauma. Additionally, war exposure was linked to decreased epigenetic ageing.

**Meaning:** Exposure to war events in refugee children and adolescents is associated with a number of distinct DNAm sites.

## Abstract

**Importance:** Exposure to war is associated with poor mental health outcomes. Adverse and traumatic experiences can lead to long-lasting DNA methylation (DNAm) changes, potentially mediating the link between adversity and mental health. To date, limited studies have investigated the impact of war on DNAm in children or adolescents, hampering our understanding of the biological impact of war exposure.

**Objective:** To identify salivary DNAm differences associated with war exposure in refugee children and adolescents.

**Design, Setting, and Participants:** 1507 Syrian refugee children and adolescent and their primary caregiver were recruited from tented settlements in Lebanon. Data collection was carried out in two waves, one year apart from October 2017 to January 2018 and October 2018 to January 2019. Children and their caregiver were interviewed and children provided saliva samples for DNA extraction (Y1: 1449, Y2:872). Data analysis was conducted in 2022 and 2023.

**Main Outcomes and Measures:** Salivary DNAm levels were assayed with Illumina-Infinium Human Methylation EPIC BeadChip and war exposure was assessed by child and caregiver questionnaires. Epigenetic ageing acceleration was estimated using a set of preexisting epigenetic ageing clocks. A literature search was conducted to identify previously reported DNAm correlates of childhood trauma.

**Results:** The study population included 1507 children and adolescents ($M_{age}$=11.3, 6-19 years old, 47.4% male). Children who reported war events had a number of differentially methylated sites and regions. Enrichment analyses indicated an enrichment of gene sets related to transmembrane transport, neurotransmission, and intracellular movement in genes that exhibited differential methylation. Sex-stratified analyses found a number of sex-specific DNAm differences associated with war exposure. Only two (out of 258) previously reported trauma- associated DNAm sites were associated with war exposure [B=-.004, 95% CI -.005 to -.003, $p_{Bonf}$= .037; B=-.005, 95% CI -.006 to -.004, $p_{Bonf}$= .026]. Any war exposure or bombardment was nominally associated with decreased

epigenetic age using Horvath's multi-tissue clock [B=-.39, 95% CI -.63 to -.14; p = .007; B=-.42, 95% CI -.73 to -.11; p = .002].

**Conclusions and Relevance:** In this cohort of Syrian refugee children and adolescents, war exposure is associated with a small number of distinct differences in salivary DNAm.

## Introduction

As of 2023, it was estimated that 117.2 million people had been forcibly displaced worldwide [1]. This population includes a substantial number who had fled countries due to armed conflict [2]. Notably, a large number of these displaced individuals are children [3], who are at an elevated risk of poor mental health often linked to the experience of premigration war [4,5]. Symptoms of psychological distress are common and there is a need to better understand the link between war exposure and subsequent mental health [6,7].

Numerous human studies have found that adverse life experiences, ranging from inadequate maternal care to war exposure, can modify biological processes including DNA methylation (DNAm) [8–11]. DNAm is an epigenetic process whereby cytosine-guanine (CpG) dinucleotide sites are chemically modified throughout the genome [11,12]. DNAm can control the expression of nearby genes and is a potential mediator between adversity and psychiatric outcomes [13,14]. Early investigations primarily adopted a candidate gene approach, targeting genes involved in key psychiatric pathways, and identified DNAm correlates of trauma and adversity [15–17]. More recent, hypothesis-free epigenome-wide association studies (EWAS) have identified DNAm differences although these rarely overlap with candidate loci [18–20]. In addition to studying independent DNAm loci, biological or epigenetic ageing can be inferred from DNAm levels at distinct sets of CpGs [21–23]. Epigenetic ageing is frequently exacerbated following adversity [10,24,25].

To date, there has been limited research concerning the impact of war on DNAm and the majority concentrates on military personnel [26–28]. Civilian populations are rarely studied and findings are inconclusive, however, war exposure has been consistently associated with global decreases in DNAm [29–31]. Most research has been conducted with a small cohort of mother-infant dyads from the Democratic Republic of the Congo. War exposure was associated with DNAm in genes associated with neuronal plasticity (*BDNF*), and stress responses (*CRH, CRHBP, NR3C1 and FKBP5*) in various tissues [32,33]. Prenatal war exposure was also associated with DNAm in the *NR3C1* promoter in placental tissue, but not in maternal blood and vice versa for *IGF1* and *IGF2* [29]. Conversely, a study in older adults found no association between childhood war exposure and *BDNF* DNAm [34]. A more

recent EWAS on the Congolese dyads found a small number of DNAm differences associated with war exposure in both mother and infant as well as accelerated epigenetic aging in infants [35].

While there is evidence that many forms of adversity during childhood and adolescence can impact DNAm, to our knowledge only a single study has investigated the impact of war exposure in this age group [36]. This study in Burundian refugee children was unable to detect any buccal DNAm differences following war exposure, however the authors note that it was not adequately powered to detect small effects. Building on this research, we aimed to comprehensively assess the DNAm differences associated with war exposure in a sample of Syrian refugee children and adolescents. We aimed to identify differentially methylated probes (DMPs) and regions (DMRs) associated with war exposure in a hypothesis-free manner. In addition, we attempted to replicate previously published DMPs associated with traumatic or adverse experiences in childhood, hypothesising that these would also be associated with war exposure. Finally, we tested whether epigenetic age acceleration was associated with war exposure, hypothesising that war would accelerate epigenetic ageing.

## Methods

This set of analyses has been pre-registered [37]. Deviations or additions to the pre-registered analysis plan are described. Full methods are detailed in the eMethods.

## Sample

This study uses a subset of a longitudinal cohort study of 1600 Syrian refugee children and their primary caregivers from informal tented settlements in the Beqaa region of Lebanon (Table 1) [38]. Ethical approval was granted by the Institutional Review Board of the University of Balamand/Saint George Hospital University Medical Center, Lebanon (ref: IRB/O/024-16/1815). The study was reviewed by the Lebanese National Consultative Committee on Ethics and approved by the Ministry of Public Health. Caregivers provided written informed consent and children provided assent. Interview data and saliva samples were collected by trained Arabic-speaking interviewers across two study waves, one year apart, with 1007 pairs followed up (October 2017-January 2019). Exclusions resulted in 1449 year 1 and 872 year 2 participants (1507 overall).

## Measures

### *DNAm*

Saliva was collected with Genefix saliva collection tubes (Isohelix). DNAm profiling at 865,859 sites was conducted on salivary DNA with the Infinium MethylationEPIC v1.0 BeadChip kit (Illumina, Inc., San Diego, CA). Samples from different waves were analysed concurrently and underwent a series of quality control and normalisation steps (eMethods).

Epigenetic ageing was assessed using a range of DNAm clocks which were selected for their prevalence in the literature (Horvath multi-tissue [22], Skin and Blood [39], Hannum [40], GrimAge [21] and PhenoAge [41]) or their relevance to paediatric populations (PedBE [42] and Wu clocks [43]; eTable 1) due to the absence of clocks developed specifically for salivary DNA in children. DunedinPACE was also included due to the use of longitudinal sampling during development [44].

### *Interview data*

War exposure was measured using the War Events Questionnaire (WEQ), a 25-item checklist of war events [45]. We assessed the cumulative effect of war exposure, the impact of different types of exposure, and contrary to the pre-registered plan the impact of any war exposure (eTable 2). Age, sex, nationality, smoking status, time since leaving Syria, pubertal status, body mass index (BMI) and the quality of the current living environment were recorded. Genome-wide genotyping data was also derived from salivary DNA.

## Statistical analyses

Analyses were conducted using R (v 4.2.2) in RStudio. DMPs associated with war exposure were identified by constructing probe-wise linear models with Limma [46], restricted to variable autosomal probes with >5% β-value ranging between the 10th and 90th percentile across all individuals (544,587 probes). Bonferroni-corrected $\alpha=0.05$ was used as a significance threshold. Gene set enrichment analyses were performed using the *methylglm* function from the methylGSA package [47], which accounts for the unequal number of probes per gene without the need to specify a p-value cutoff. Enriched gene sets were considered where $p_{FDR}<.05$. DMRs were identified with DMRcate where $p_{Stouffer}<.05$ [48].

Replication analyses were conducted on CpGs which had been previously associated with traumatic experiences in childhood. Probe-wise linear mixed effect models were fitted using the lme4 package [49] utilising the repeated samples for each individual where available. We considered successful replication where the difference in DNAm was in the same direction as the original report and $p_{Bonferonni}<.05$.

Epigenetic age analyses were only conducted on those with the highest confidence in the accuracy of chronological age (Y1: n=994, Y2: 571). For each clock, epigenetic age acceleration was calculated as the residual of a linear mixed effect model which fitted epigenetic age onto chronological age, alongside covariates. Analyses tested the corrected residuals within linear mixed effect models with war exposure and sex as fixed effects and the donor as a random effect.

# Results

## Epigenome-wide DNA methylation differences in war-exposed children

We first aimed to identify probes and regions of the genome that were differentially methylated with exposure to war in a hypothesis-free manner. We found that children who reported war events that impacted their home exhibited increased DNAm at cg18691565 ($p_{Bonf}$=.023; *ACBD5*), those that reported any war exposure exhibited reduced methylation at cg08095654 ($p_{Bonf}$=.034) and cg03806695 ($p_{Bonf}$=.003), and those that reported other forms of war-related violence exhibited reduced methylation at cg14747961 ($p_{Bonf}$=.0.031; *SCAMP5*; Figure 1; eTable 3, eFigure 2). The number of war-related events experiences was positively associated with methylation at cg17049090 ($p_{Bonf}$=0.042, *ADGRB2*). No inflation in the test statistic was observed when considering any war exposure or violence in the home ($\lambda$ = .99, .95; eFigure 3). However there was some evidence for inflation when considering other war-related violence or total war events, suggesting caution when interpreting this result ($\lambda$ = 1.19, 1.14). Follow-up analyses indicated that these DNAm differences were not confounded by analysis batch, time since leaving Syria, pubertal status, BMI, the quality of the current living environment or population stratification, nor were they biased by genetic relatedness across the sample (eTable 4).

Exploratory enrichment analyses of the results were performed to identify pathways or biological functions that are overrepresented in the top DMPs. These indicated an enrichment of DNAm differences in gene sets related to transmembrane transport (*amino acid:sodium symporter activity*) associated with total war exposure, protein degradation (*proteasome accessory complex*) in those that experience bombardment, and neurotransmission (*glutamate receptor activity*) and intracellular movement (*microfilament motor activity*) in those that experienced violence to a close individual (Figure 2, eTable 5). We only identified a significant DMR in those that reported any war exposure (19: 58728390-58728865; Figure 1A; eTable 6). This DMR contained four of the top 15 DMPs associated with any war exposure, however it hasn't been annotated to a gene.

Sex-stratified analyses were performed to identify sex-specific DMPs and DMRs, as well as those located on the sex chromosomes. In females, cg09582238 (*PERM1*) was associated with

bombardment, cg18518909 (*ATP6V1H*) was associated with other forms of violence, cg20560283 was associated with violence in the home and six CpGs (cg12801791, TESC; cg00588499; cg18251449; cg25844655; cg11571585; cg11571585, GSX1) were associated with any war exposure (eTable 7). In males, cg11337624 was correlated with total war exposure, cg13647973 was associated with any war exposure and three CpGs (cg13647973; cg22321036; cg04673462) were associated with bombardment (eTable 8). The majority of these DMPs exhibited significant interactions with sex when considering the entire cohort. In females, exposure to any war-related events was associated with four DMRs (Chr19: 58728390-58728865; Chr17: 21187376-21187420; Chr2: 120516537-120516628; Chr7: 63386226-63387147, *RP11-73B2.6*; eTable 9), while bombardment was associated with a single DMR (Chr4: 124232-125504, *ZNF718*). In males, exposure to bombardment was associated with a single DMR (Chr1: 38460950-38461896).

## Trauma-associated DNA methylation differences in war-exposed children

We next aimed to replicate existing trauma-associated DMPs. We identified a total of 267 (258 autosomal) CpGs from the literature that were associated with potentially traumatic experiences in childhood. Of these, only two CpGs (cg24244000 & cg05717473) were associated with war exposure (violence in the home and other forms of violence) in the current sample after correction for multiple-testing (B=-.004, 95% CI -.005 to -.003, p=.00014, $p_{Bonf}$= .037; B=-.005, 95% CI -.006 to -.004, p=.00010, $p_{Bonf}$= .026; Figure 3). Furthermore, most were not nominally significant (4.6% p<.05) and of those that were, only 43% had an identical direction of effect to the original report (eTable 11).

In females, a single CpG (cg10091102) exhibited reduced DNAm in those that reported any war exposure (B=-.008, 95% CI -.010 to -.007, p=.000006, $p_{Bonf}$= .0017) or bombardment (B=-.006, 95% CI -.007 to -.005, p=.00002, $p_{Bonf}$= .0047; eFigure 4A), however the direction of effect opposed that of the original report [50]. There were no replicated DMPs in males (eFigure 4B).

## Epigenetic ageing differences in war-exposed children

Finally, we investigated the impact of war exposure upon age acceleration using commonly used epigenetic ageing clocks. Horvath's multi-tissue clock indicated reduced epigenetic ageing in those

that reported bombardment-related events (B=-.39, 95% CI -.63 to -.14; p = .002) or any war exposure (B=-.42, 95% CI -.73 to -.11; p = .007; Figure 4). However, these do not survive when applying correction for multiple testing using the estimated effective number of tests estimated due to non-independence ($p_{Bonf}$=.081; $p_{Bonf}$=..259 respectively). These results were similar when controlling for a number of potential confounders (eFigure 5). When clocks developed in children were applied to the least developmentally advanced of the cohort, age acceleration using Horvath's clock was again negatively associated with bombardment (B=-.37, 95% CI -.70 to -.63; p = .006) and any war exposure (B=-.37, 95% CI -.70 to -.05; p = .024; eFigure 6).

Sex-stratified analyses revealed similar associations between war exposure and ageing but differing indicative clocks (eFigure 7). In females, exposure to any war-related events (B=-.52, 95% CI -.91 to -.13; p = .010) and bombardment (B=-.38, 95% CI -.70 to -.07; p = .017) was associated with decreased age-acceleration according to Horvath's clock, whereas bombardment was associated with decreased ageing according to Wu's clock in males (B=-.44, 95% CI -.83 to -.04; p = .029). Despite these differences, there were no significant interactions with sex.

## Discussion

Our epigenome-wide approach revealed a small number of DMPs and DMRs associated with total war exposure, any war exposure, violence in the home and other forms of war-related violence. To our knowledge, DNAm at these loci have not been previously associated with similar exposures, although one CpG is annotated to a gene which has been shown to modulate susceptibility to depressive-like behaviours in a mouse model *(ADGRB2)* [51].

We also identified a number of sex-specific DMPs which have been associated with ageing (cg18518909, cg20560283, cg12801791, cg18251449) or annotated to genes associated with Gulf War illness (*TESC*, *ATP6V1H)* [52] or neuronal development (*GSX1*) [53]. Gene enrichment analyses suggested an over-representation of DNAm differences in genes associated with transmembrane transport and neurotransmission amongst others. However, caution is required given that many were associated with war exposure variables lacking significant DMPs and DMRs. Additionally, the biological significance of any identified DNAm differences is unclear (Δ2.4%, 4.3% and 2.0% for the main binary DMPs). While DNAm differences below 5% are considered small, these are in line with existing studies looking at the impact of environmental adversities within peripheral tissues [54,55]. Furthermore, they are only small when considering the cell population as a whole. At a cellular level, the difference between unmethylated and methylated may have significant impacts on that cell's functioning [56]. This is particularly relevant when considering heterogeneous tissues like saliva. While enrichment analyses provide some clue as to the downstream impacts of such DNAm differences, further multi-omic or functional investigation would be needed to elucidate the true outcomes.

Due to a lack of similar cohorts for replication, we attempted to replicate previously published CpGs associated with childhood traumatic experiences. There was little evidence that these were associated with war exposure in our cohort with only two DMPs surviving multiple testing corrections. There are various reasons why replication failed. Firstly, research has focused predominately on white European populations. Some adversity-associated DNAm loci are genotype-dependent [57–59], potentially driving population-level disparities. Secondly, war exposure may represent a unique form of adversity. Existing research tends to examine a broad set of adversities which includes more common and less

life-threatening events such as bullying or poverty. Finally, this study measured DNAm in saliva which is a highly heterogeneous tissue and rarely used within the studies identified for replication. While adversity-associated DNAm differences may be mirrored across multiple tissues, they can differ significantly [18].

Despite the selected epigenetic clocks having been trained predominately in adult populations in tissues other than saliva, many were moderately correlated with chronological age. Interestingly, the two clocks (Horvath & Wu) which were nominally associated with war exposure, were some of the few clocks developed using paediatric samples indicating the importance of carefully selecting the most appropriate clock for the study population and tissue. Consequently, these clocks had marginally better agreement with chronological age, going some way to explain these findings. Furthermore, Horvath's muti-tissue clock was developed on a particularly large sample of 51 healthy tissues and cell types allowing its application to multiple tissues. Wu's clock was developed on a predominantly male sample [43], potentially explaining why it was only associated with war exposure in males.

Contrary to our hypotheses and most existing research [60–63], we found that war exposure was nominally associated with decreased epigenetic ageing. However, a recent study suggests that the direction of adversity-associated ageing may differ within populations with a relatively high burden of adversity such as care leavers [64]. Similarly, the BIOPATH cohort has been forcibly displaced and are subject to continued adversity [5,65]. These are important to consider when accumulation of stressors and more immediate factors in the lives of refugees may have a greater impact on mental health [66,67]. Alternatively, earlier exposure to war may influence their current environments (e.g., increased parental support), tempering epigenetic age acceleration [68–70].

It should be noted that "ageing" is an unclear concept within younger populations. While in adults epigenetic ageing is considered detrimental and is associated with degeneration and mortality, in children it may simply represent development [71]. Accordingly, our preliminary findings may suggest that war exposure is associated with delayed development in similar manner to the observed association between traumatic experiences and delayed physical and cognitive development [72–74]. However, it should be emphasised that the observed age deceleration did not survive multiple

correction and that, in general, epigenetic clocks performed poorly within this cohort. Future replication or the development of better performing epigenetic clocks for our samples may help support this finding.

This study has some limitations. Despite attempts to capture the "severity" of war exposure through the use of various war exposure measures, it is likely this approach does not fully appreciate the complexity of war. It does not capture the repetition or chronicity of each war event, nor does it capture the subjective assessment of the individual. Furthermore, we have no information on the timing of such events which precludes investigating the stability of any DNAm differences identified here. Secondly, the wide age range of our sample as well as the differences in timing of exposure may hinder the identification of DNAm differences which are age or timing-specific. Finally, our sample contains a relatively small number of individuals who reported no war exposure.

### *Conclusion*

To our knowledge this is only the second study to examine the impact of postnatal war exposure upon DNAm in children or adolescents and one of only a few investigations in a civilian cohort. In addition to furthering our understanding of the biological impact of war this study also provides much-needed research on under-researched populations outside of Europe and North America. War exposure was associated with various differences in DNAm in Syrian refugee children and adolescents supporting the biological embedding of negative life events. There is little evidence that these overlap with previously reported adversity-associated DNAm phenotypes, but findings suggest that war-associated adversity in children and adolescents may delay development.

## References


1. Global Appeal 2023. Global Focus. Accessed January 7, 2024. https://reporting.unhcr.org/globalappeal-2023

2. Figures at a glance. UNHCR UK. Accessed August 20, 2023. https://www.unhcr.org/uk/about-unhcr/who-we-are/figures-glance

3. Child Displacement and Refugees. UNICEF DATA. Accessed August 20, 2023. https://data.unicef.org/topic/child-migration-and-displacement/displacement/

4. Brandt L, Henssler J, Müller M, Wall S, Gabel D, Heinz A. Risk of Psychosis Among Refugees: A Systematic Review and Meta-analysis. *JAMA Psychiatry*. 2019;76(11):1133-1140. doi:10.1001/jamapsychiatry.2019.1937

5. Guo L, Li L, Xu K, et al. Characterization of Premigration and Postmigration Multidomain Factors and Psychosocial Health Among Refugee Children and Adolescents After Resettlement in Australia. *JAMA Network Open*. 2023;6(4):e235841. doi:10.1001/jamanetworkopen.2023.5841

6. Bürgin D, Anagnostopoulos D, Vitiello B, Sukale T, Schmid M, Fegert JM. Impact of war and forced displacement on children's mental health—multilevel, needs-oriented, and trauma-informed approaches. *Eur Child Adolesc Psychiatry*. 2022;31(6):845-853. doi:10.1007/s00787-022-01974-z

7. Frounfelker RL, Miconi D, Farrar J, Adam Brooks M, Rousseau C, Betancourt TS. Mental Health of Refugee Children and Youth: Epidemiology, Interventions and Future Directions. *Annu Rev Public Health*. 2020;41:159-176. doi:10.1146/annurev-publhealth-040119-094230

8. Vinkers CH, Kalafateli AL, Rutten BPF, et al. Traumatic stress and human DNA methylation: a critical review. *Epigenomics*. 2015;7(4):593-608. doi:10.2217/epi.15.11

9. Szyf M. The epigenetics of perinatal stress . *Dialogues Clin Neurosci*. 2019;21(4):369-378. doi:10.31887/DCNS.2019.21.4/mszyf

10. Wolf EJ, Maniates H, Nugent N, et al. Traumatic Stress and Accelerated DNA Methylation Age: A Meta-Analysis. *Psychoneuroendocrinology*. 2018;92:123-134. doi:10/gdnrjj

11. Aristizabal MJ, Anreiter I, Halldorsdottir T, et al. Biological embedding of experience: A primer on epigenetics. *PNAS*. 2020;117(38):23261-23269. doi:10.1073/pnas.1820838116

12. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*. 2016;17(8):487-500. doi:10.1038/nrg.2016.59

13. Copeland WE, Shanahan L, Hinesley J, et al. Association of Childhood Trauma Exposure With Adult Psychiatric Disorders and Functional Outcomes. *JAMA Network Open*. 2018;1(7):e184493. doi:10.1001/jamanetworkopen.2018.4493

14. Thumfart KM, Jawaid A, Bright K, Flachsmann M, Mansuy IM. Epigenetics of childhood trauma: Long term sequelae and potential for treatment. *Neurosci Biobehav Rev*. 2022;132:1049-1066. doi:10.1016/j.neubiorev.2021.10.042

15. Harms MB, Birn R, Provencal N, et al. Early life stress, FK506 binding protein 5 gene (FKBP5) methylation, and inhibition-related prefrontal function: A prospective longitudinal study. *Dev Psychopathol*. 2017;29(5):1895-1903. doi:10.1017/S095457941700147X

16. Peng H, Zhu Y, Strachan E, et al. Childhood Trauma, DNA Methylation of Stress-Related Genes, and Depression: Findings From Two Monozygotic Twin Studies. *Psychosom Med*. 2018;80(7):599-608. doi:10.1097/PSY.0000000000000604

17. Zou Z, Huang Y, Wang J, Min W, Zhou B. DNA methylation of IL-4 gene and the association with childhood trauma in panic disorder. *Psychiatry Res*. 2020;293:113385. doi:10.1016/j.psychres.2020.113385

18. Kandaswamy R, Hannon E, Arseneault L, et al. DNA methylation signatures of adolescent victimization: analysis of a longitudinal monozygotic twin sample. *Epigenetics*. 2021;16(11):1169-1186. doi:10.1080/15592294.2020.1853317

19. Marzi SJ, Sugden K, Arseneault L, et al. Analysis of DNA Methylation in Young People: Limited Evidence for an Association Between Victimization Stress and Epigenetic Variation in Blood. *Am J Psychiatry*. 2018;175(6):517-529. doi:10.1176/appi.ajp.2017.17060693

20. Houtepen LC, Hardy R, Maddock J, et al. Childhood adversity and DNA methylation in two population-based cohorts. *Transl Psychiatry*. 2018;8. doi:10.1038/s41398-018-0307-3

21. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY)*. 2019;11(2):303-327. doi:10.18632/aging.101684

22. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;14(10):R115. doi:10.1186/gb-2013-14-10-r115

23. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nature Reviews Genetics*. 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3

24. Boks MP, van Mierlo HC, Rutten BPF, et al. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. *Psychoneuroendocrinology*. 2015;51:506-512. doi:10.1016/j.psyneuen.2014.07.011

25. Clausing ES, Binder AM, Non AL. Epigenetic age associates with psychosocial stress and resilience in children of Latinx immigrants. *Epigenomics*. 2021;13(21):1677-1699. doi:10.2217/epi-2019-0343

26. Hossack MR, Reid MW, Aden JK, Gibbons T, Noe JC, Willis AM. Adverse Childhood Experience, Genes, and PTSD Risk in Soldiers: A Methylation Study. *Mil Med*. 2020;185(3-4):377-384. doi:10.1093/milmed/usz292

27. Snijders C, Maihofer AX, Ratanatharathorn A, et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clinical Epigenetics*. 2020;12(1):11. doi:10.1186/s13148-019-0798-7

28. Straight B, Fisher G, Needham BL, et al. Lifetime stress and war exposure timing may predict methylation changes at NR3C1 based on a pilot study in a warrior cohort in a small-scale society in Kenya. *Am J Hum Biol*. 2021;33(4):e23515. doi:10.1002/ajhb.23515

29. Rodney NC, Mulligan CJ. A biocultural study of the effects of maternal stress on mother and newborn health in the Democratic Republic of Congo. *Am J Phys Anthropol*. 2014;155(2):200-209. doi:10.1002/ajpa.22568

30. Clukay CJ, Hughes DA, Rodney NC, Kertes DA, Mulligan CJ. DNA methylation of methylation complex genes in relation to stress and genome-wide methylation in mother-newborn dyads. *Am J Phys Anthropol*. 2018;165(1):173-182. doi:10.1002/ajpa.23341

31. Montoya-Williams D, Quinlan J, Clukay C, Rodney NC, Kertes DA, Mulligan CJ. Associations between maternal prenatal stress, methylation changes in IGF1 and IGF2, and birth weight. *J Dev Orig Health Dis*. 2018;9(2):215-222. doi:10.1017/S2040174417000800

32. Kertes DA, Bhatt SS, Kamin HS, Hughes DA, Rodney NC, Mulligan CJ. BNDF methylation in mothers and newborns is associated with maternal exposure to war trauma. *Clin Epigenetics*. 2017;9:68. doi:10.1186/s13148-017-0367-x

33. Kertes DA, Kamin HS, Hughes DA, Rodney NC, Bhatt S, Mulligan CJ. Prenatal Maternal Stress Predicts Methylation of Genes Regulating the Hypothalamic-Pituitary-Adrenocortical System in Mothers and Newborns in the Democratic Republic of Congo. *Child Dev*. 2016;87(1):61-72. doi:10.1111/cdev.12487

34. Zhou A, Ancelin ML, Ritchie K, Ryan J. Childhood adverse events and BDNF promoter methylation in later-life. *Front Psychiatry*. 2023;14:1108485. doi:10.3389/fpsyt.2023.1108485

35. Quinn EB, Hsiao CJ, Maisha FM, Mulligan CJ. Prenatal maternal stress is associated with site-specific and age acceleration changes in maternal and newborn DNA methylation. *Epigenetics*. 18(1):2222473. doi:10.1080/15592294.2023.2222473

36. Mattonet K, Scharpf F, Block K, Kumsta R, Hecker T. No association between war-related trauma or PTSD symptom severity and epigenome-wide DNA methylation in Burundian refugees. *Eur J Psychotraumatol*. 2023;14(2):2228155. doi:10.1080/20008066.2023.2228155

37. Smeeth D, McEwen F, Popham C, et al. Associations between salivary DNA methylation and war exposure in Syrian refugee children and adolescents. September 14, 2022. Accessed August 1, 2023. https://osf.io/6u27r

38. McEwen FM, Popham C, Moghames P, et al. Cohort profile: biological pathways of risk and resilience in Syrian refugee children (BIOPATH). *Soc Psychiatry Psychiatr Epidemiol*. Published online January 18, 2022. doi:10.1007/s00127-022-02228-8

39. Horvath S, Oshima J, Martin GM, et al. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. *Aging (Albany NY)*. 2018;10(7):1758-1775. doi:10.18632/aging.101508

40. Hannum G, Guinney J, Zhao L, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. *Molecular Cell*. 2013;49(2):359-367. doi:10.1016/j.molcel.2012.10.016

41. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)*. 2018;10(4):573-591. doi:10.18632/aging.101414

42. McEwen LM, O'Donnell KJ, McGill MG, et al. The PedBE clock accurately estimates DNA methylation age in pediatric buccal cells. *PNAS*. Published online October 11, 2019. doi:10.1073/pnas.1820843116

43. Wu X, Chen W, Lin F, et al. DNA methylation profile is a quantitative measure of biological aging in children. *Aging (Albany NY)*. 2019;11(22):10031-10051. doi:10.18632/aging.102399

44. Belsky DW, Caspi A, Corcoran DL, et al. DunedinPACE, a DNA methylation biomarker of the pace of aging. Deelen J, Tyler JK, Suderman M, Deelen J, eds. *eLife*. 2022;11:e73420. doi:10.7554/eLife.73420

45. Karam EG, Al-Atrash R, Saliba S, Melhem N, Howard D. The War Events Questionnaire. *Soc Psychiatry Psychiatr Epidemiol*. 1999;34(5):265-274. doi:10.1007/s001270050143

46. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*. 2015;43(7):e47. doi:10.1093/nar/gkv007

47. Ren X, Kuan PF. methylGSA: a Bioconductor package and Shiny app for DNA methylation data length bias adjustment in gene set testing. *Bioinformatics*. 2019;35(11):1958-1959. doi:10.1093/bioinformatics/bty892

48. Peters TJ, Buckley MJ, Statham AL, et al. De novo identification of differentially methylated regions in the human genome. *Epigenetics & Chromatin*. 2015;8(1):6. doi:10.1186/1756-8935-8-6

49. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 2015;67(1):1-48. doi:10.18637/jss.v067.i01

50. Engdahl E, Alavian-Ghavanini A, Forsell Y, Lavebratt C, Rüegg J. Childhood adversity increases methylation in the GRIN2B gene. *J Psychiatr Res*. 2021;132:38-43. doi:10.1016/j.jpsychires.2020.09.022

51. Okajima D, Kudo G, Yokota H. Antidepressant-like behavior in brain-specific angiogenesis inhibitor 2-deficient mice. *J Physiol Sci*. 2011;61(1):47-54. doi:10.1007/s12576-010-0120-0

52. Trivedi MS, Abreu MM, Sarria L, et al. Alterations in DNA Methylation Status Associated with Gulf War Illness. *DNA Cell Biol*. 2019;38(6):561-571. doi:10.1089/dna.2018.4469

53. Finkel Z, Esteban F, Rodriguez B, et al. AAV6 mediated Gsx1 expression in neural stem progenitor cells promotes neurogenesis and restores locomotor function after contusion spinal cord injury. *Neurotherapeutics*. 2024;21(4):e00362. doi:10.1016/j.neurot.2024.e00362

54. Melas PA, Wei Y, Wong CCY, et al. Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. *Int J Neuropsychopharmacol*. 2013;16(7):1513-1528. doi:10.1017/S1461145713000102

55. Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. *PLoS One*. 2012;7(1):e30148. doi:10.1371/journal.pone.0030148

56. Breton CV, Marsit CJ, Faustman E, et al. Small-Magnitude Effect Sizes in Epigenetic End Points are Important in Children's Environmental Health Studies: The Children's Environmental Health

and Disease Prevention Research Center's Epigenetics Working Group. *Environ Health Perspect*. 2017;125(4):511-526. doi:10.1289/EHP595

57. Czamara D, Tissink E, Tuhkanen J, et al. Combined effects of genotype and childhood adversity shape variability of DNA methylation across age. *Transl Psychiatry*. 2021;11(1):1-11. doi:10.1038/s41398-020-01147-z

58. Lam D, Ancelin ML, Ritchie K, Freak-Poli R, Saffery R, Ryan J. Genotype-dependent associations between serotonin transporter gene (SLC6A4) DNA methylation and late-life depression. *BMC Psychiatry*. 2018;18(1):282. doi:10.1186/s12888-018-1850-4

59. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci*. 2013;16(1):33-41. doi:10.1038/nn.3275

60. Marini S, Davis KA, Soare TW, et al. Adversity exposure during sensitive periods predicts accelerated epigenetic aging in children. *Psychoneuroendocrinology*. 2020;113:104484. doi:10.1016/j.psyneuen.2019.104484

61. Tang R, Howe LD, Suderman M, Relton CL, Crawford AA, Houtepen LC. Adverse childhood experiences, DNA methylation age acceleration, and cortisol in UK children: a prospective population-based cohort study. *Clin Epigenetics*. 2020;12(1):55. doi:10.1186/s13148-020-00844-2

62. Lawn RB, Anderson EL, Suderman M, et al. Psychosocial adversity and socioeconomic position during childhood and epigenetic age: analysis of two prospective cohort studies. *Hum Mol Genet*. 2018;27(7):1301-1308. doi:10.1093/hmg/ddy036

63. Kim K, Yaffe K, Rehkopf DH, et al. Association of Adverse Childhood Experiences With Accelerated Epigenetic Aging in Midlife. *JAMA Netw Open*. 2023;6(6):e2317987. doi:10.1001/jamanetworkopen.2023.17987

64. Meier M, Kantelhardt S, Gurri L, et al. Childhood Trauma Is Linked to Epigenetic Age Deceleration in Young Adults with Previous Youth Residential Care Placements. Published online January 6, 2024. doi:10.31234/osf.io/merqk

65. Popham CM, McEwen FS, Karam E, et al. Predictors of psychological risk and resilience among Syrian refugee children. *Journal of Child Psychology and Psychiatry*. 2022;n/a(n/a). doi:10.1111/jcpp.13670

66. Moran JK, Jesuthasan J, Schalinski I, et al. Traumatic Life Events and Association With Depression, Anxiety, and Somatization Symptoms in Female Refugees. *JAMA Network Open*. 2023;6(7):e2324511. doi:10.1001/jamanetworkopen.2023.24511

67. Tinghög P, Malm A, Arwidson C, Sigvardsdotter E, Lundin A, Saboonchi F. Prevalence of mental ill health, traumas and postmigration stress among refugees from Syria resettled in Sweden after 2011: a population-based survey. *BMJ Open*. 2017;7(12):e018899. doi:10.1136/bmjopen-2017-018899

68. Danoff JS, Ramos EN, Hinton TD, et al. Father's care uniquely influences male neurodevelopment. *Proceedings of the National Academy of Sciences*. 2023;120(31):e2308798120. doi:10.1073/pnas.2308798120

69. Brody GH, Yu T, Chen E, Beach SRH, Miller GE. Family-centered prevention ameliorates the longitudinal association between risky family processes and epigenetic aging. *Journal of Child Psychology and Psychiatry*. 2016;57(5):566-574. doi:10.1111/jcpp.12495

70. Sullivan ADW, Bozack AK, Cardenas A, et al. Parenting Practices May Buffer the Impact of Adversity on Epigenetic Age Acceleration Among Young Children With Developmental Delays. *Psychol Sci*. 2023;34(10):1173-1185. doi:10.1177/09567976231194221

71. Simpkin AJ, Howe LD, Tilling K, et al. The epigenetic clock and physical development during childhood and adolescence: longitudinal analysis from a UK birth cohort. *International Journal of Epidemiology*. 2017;46(2):549-558. doi:10.1093/ije/dyw307

72. Prebeg Ž, Bralić I. Changes in menarcheal age in girls exposed to war conditions. *American Journal of Human Biology*. 2000;12(4):503-508. doi:10.1002/1520-6300(200007/08)12:4<503::AID-AJHB10>3.0.CO;2-H

73. Li L, Denholm R, Power C. Child maltreatment and household dysfunction: associations with pubertal development in a British birth cohort. *Int J Epidemiol*. 2014;43(4):1163-1173. doi:10.1093/ije/dyu071

74. Stenson AF, Michopoulos V, Stevens JS, Powers A, Jovanovic T. Sex-Specific Associations Between Trauma Exposure, Pubertal Timing, and Anxiety in Black Children. *Frontiers in Human Neuroscience*. 2021;15. doi:10.3389/fnhum.2021.636199

## Acknowledgements

The BIOPATH study was funded by the Eunice Shriver National Institute of Child Health & Human Development (NICHD; R01HD083387). The funder played no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication. Training was provided by the University of Michigan supported by a grant from the National Institutes of Health National Institute on Aging (NIA; R25AG053227). We warmly thank all participating families for their participation. We thank Cassandra M Popham, PhD (Queen Mary University of London), Andrew May, PhD (Queen Mary University of London), Patricia Moghames, Stephanie Legoff, Nicolas Puvis, and Zeina Hassan, and all other members of the BIOPATH team for their dedication, hard work and insights. We thank the Genome Centres at QMUL and UCL for the DNA extraction and DNA methylation analysis. This paper is dedicated to John Fayyad, who sadly passed away during the study.

Dr Smeeth and Professor Pluess had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## Figure legends

Figure 1. Manhattan plots of the epigenome-wide association studies for violence in the home, any war exposure, total war exposure and other forms of violence.

The red line indicates the Bonferroni-corrected p-value threshold. Genome-wide significant differentially methylated probes (DMPs) are in red and the only differentially methylated region is indicated by the blue vertical line.

Figure 2. Top 10 gene sets associated with war exposure.

Each gene set is labelled with the description and war exposure measure it is associated with. P-values have been FDR-corrected. Bar length represents the number of genes within that gene set.

Figure 3. Volcano plot of the regression beta values against p-values for the replication analyses.

Dashed lines represent the nominal (p=.05) and Bonferroni-corrected p-value thresholds. CpGs with p<.001 have been labelled.

Figure 4. Results for the association between epigenetic age acceleration and war exposure.

Dots represent regression coefficients and error bars represent 95% confidence intervals. Negative estimates indicate slower age acceleration and positive estimates indicate faster age acceleration. Where p<.05, the p-value is given.

## Tables

Table 1. Cohort description at baseline

| | |
|---|---|
| N | 1507 |
| Male, N (%) | 714 (47.4) |
| Age, mean (SD) | 11.3 (2.4) |
| Nationality, N (%) | |
| Syrian | 1485 (98.5) |
| Other | 22 (1.5) |
| Reported smoker, N (%) | 19 (1.3) |
| BMI, mean (SD)[a] | 17.9 (3.6) |
| Time since leaving Syria, N (%)[b] | |
| 0-12 months | 275 (18.2) |
| 12-24 months | 213 (14.1) |
| 24-36 months | 212 (14.1) |
| 36-48 months | 562 (37.3) |
| 48+ months | 239 (15.9) |
| Any war exposure, N (%) | 1462 (97.0) |
| Bombardment, N (%) | 1434 (95.2) |
| Other-directed violence, N (%) | 1160 (77.0) |
| Violence directed towards a close person, N (%) | 853 (56.6) |
| War-related violence in the home, N (%) | 995 (66.0) |
| Personal harm, N (%) | 453 (30.1) |
| Total war exposure, mean (SD) | 9.6 (5.5) |

[a] 643 missing BMI; [b] 6 missing time since leaving Syria;