Epigenetic impact of a 1-week intensive multimodal group program for adolescents with multiple adverse childhood experiences

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Adverse childhood experiences (ACEs, i.e., abuse, neglect, household dysfunction) represent a potential risk factor for a wide range of long-lasting diseases and shorter life expectancy. We recently described a 1-week residential group program, based on mindfulness training, artistic expression and EMDR group therapy, that significantly reduced PTSD-related symptoms and increased attention/awareness-related outcomes in adolescent girls with multiple ACEs in a randomized controlled study. Since epigenetic mechanisms (i.e., DNA methylation) have been associated with the long-lasting effects of ACEs, the present report extends these prior findings by exploring genome-wide DNA methylation changes following the program. Saliva samples from all participants (n = 44) were collected and genomic DNA was extracted prior (T1) and following (T2) the intervention. Genome-wide DNA methylation analysis using the MethylationEPIC beadchip array (Illumina) revealed 49 differentially methylated loci (DML; \( p \text{ value} < 0.001; \text{methylation change} > 10\% \)) that were annotated to genes with roles in biological processes linked to early childhood adversity (i.e., neural, immune, and endocrine pathways, cancer and cardiovascular disease). DNA sequences flanking these DML showed significant enrichment of transcription factor binding sites involved in inflammation, cancer, cardiovascular disease, and brain development. Methylation changes in SIRT5 and TRAPPC2L genes showed associations with changes in trauma-related psychological measures. Results presented here suggest that this multimodal group program for adolescents with multiple victimization modulates the DNA methylome at sites of potential relevance for health and behavioral disorders associated with ACEs.

The exposure to chronic and severe negative life experiences during early childhood is associated with the development of a host of physical and mental health problems later in life1. Adverse childhood experiences (ACEs) include physical, sexual and verbal abuse, physical and emotional neglect, witnessing violence at home, a family member suffering from addictions, mental health issues or incarcerated, and losing a parent to separation, divorce or other reason2. Children who have experienced four or more ACEs are more likely to develop long lasting health issues such as diabetes, heart disease, overweight or obesity, cancer, respiratory disease, mental health conditions, alcohol and drug abuse, interpersonal and self-directed violence and sexual risk taking3.

There is growing evidence suggesting that epigenetic modulation is one of the molecular mechanisms through which stressors interact with the genome. Epigenetic information regulates gene expression and, although
relatively stable, the epigenetic landscape is highly sensitive to environmental exposures. DNA methylation is one of the most widely studied epigenetic modifications in which a methyl group is added to a cytosine residue, most commonly in the context of cytosine–guanine dinucleotides (CpG). Children exposed to severe adversity show DNA methylation changes in genes involved in the vulnerability to stress, neurotransmission, inflammatory responses and behavior. Negative childhood exposures can trigger DNA methylation changes in genes that modulate anxiety and related phenotypes, such as the oxytocin receptor, glucocorticoid receptor, serotonin transporter gene, brain-derived neurotrophic factor and glutamate receptor. Early-life maternal and paternal stressors are predictive of DNA methylation changes detected in adolescents and both ACEs and DNA methylation changes at the glucocorticoid receptor gene have been associated with increased risk of psychopathologies during adolescence. Moreover, adverse experiences have been associated with an accelerated biological aging. The deviation between the DNA methylation age and the chronological age is a measure of the epigenetic aging rate. In children, the Pediatric-Buccal-Epigenetic (PedBE) clock is a tool to measure the biological age, providing an understanding of the environmental exposures that might influence child health and disease. Recent findings show that psychologically adverse or violent home environments can accelerate epigenetic aging in youth. Similarly, neighborhood violence or elevated parental depressive symptoms have been associated with both emotional distress and accelerated epigenetic aging in children. Importantly, an accelerated rate of epigenetic aging predicts the risk of many chronic conditions such as obesity, cancer, Alzheimer’s disease, cardiovascular disease, and all-cause mortality risk.

Recent research shows that positive childhood experiences predict positive outcomes in long-term health and can also neutralize the negative impact of ACEs on adult health. In this context, interventions to increase awareness and understanding of childhood adversities and to promote family connection have been proposed as strategies to influence health and well-being later in life. In addition, multimodal programs that combine several approaches such as cognitive behavioral therapy, exercise, yoga, music, art, EMDR (Eye Movement Desensitization and Reprocessing) therapy, individual counselling and interactions with animals have been proposed to improve wellbeing and mental health in child victims of multiple ACEs. Notably, in rodents, an enriched environmental model, which includes cognitive, somatosensory, motor and visual stimulation, reduces the negative psychological and behavioral consequences of early adversity by modulating trauma-sensitive epigenetic marks and improving neurogenesis and synaptic plasticity.

We recently described the protocol and mental health impact of a 1-week multimodal intervention group (n = 44 girls) program for adolescents (aged 13–16 years) reporting 4 or more ACEs. After completing the program, the intervention group showed significant reduction in trauma-related outcomes (~ 73% in the Short PTSD Rating Interview (SPRINT) scale; ~ 26% in the Child PTSD Symptom Scale (CPSS)) and a 57% improvement in attention/awareness-related outcomes Mindful Attention Awareness Scale-Adolescents (MAAS-A). This program addresses trauma through evidence-based therapeutic approaches, in an enriched environment that provides social, somatosensory and cognitive stimulation. Based on the literature discussed above, we hypothesize that these conditions may trigger DNA methylation changes in genes involved in the pathophysiology of multiple ACEs, such as vulnerability to stress, neurotransmission, inflammatory responses, behavior and cell aging. We hypothesize that some of the DNA methylation changes may correlate with the mental health improvements that we have previously reported in the same sample, providing insights for future mechanistic research. To test starting this hypothesis, we profiled genome-wide DNA methylation levels in saliva samples from control and intervention group participants, at baseline (T1) and post-intervention (T2), in order to detect potential physiological relevant DNA methylation changes.

**Results**

**Intensive multimodal 1-week group program causes genome-wide alterations in DNA methylation.** To identify the impact of the intervention on DNA methylation levels at each CpG on the Human MethylationEPIC array (N = > 850,000 sites), we used an ANCOVA model adjusting for DNA methylation level at baseline (T1), BMI, age, ACEs score and cell type proportions (see details in “Methods”). This approach revealed that 49 DML exhibited a p value < 0.001 and a change in DNA methylation level greater than 10% (Table 1), while 195 DML showed a p value < 0.001 and a change in DNA methylation level greater than 5% (Supplementary Table S1).

Out of the 49 DML, 87% showed an increase in DNA methylation level from baseline to post-treatment and 37 DML reside in known genes. These 49 DML were distributed across all human chromosomes except the Y chromosome and were most often found within gene bodies (57%), followed by 5’ untranslated regions (27%), and gene promoter regions of genes (up to 1500 basepairs upstream of the gene transcription start site) (16%) (Fig. 1b). Most of the DML were in open sea regions (more than 4 kb from a CpG island) (64%) and 12% were located within CpG islands (Fig. 1c). Considering the probe locations included on the array, the genomic region and location enrichments of the DML were not significant (p value > 0.05).

**Functional roles of intervention-sensitive DML.** Using a meta-database restricted to the 49 DMLs to identify molecular interactions for network biology (ConsensusPathDB-human tool), we conducted a pathway analysis and found a significant enrichment of functional interactions associated with the nervous, endocrine, immune systems, and processes involved in cancer, diabetes and cardiovascular disease (top 20 pathways with FDR q-value < 0.03, Table 2; all pathways with FDR q-value ≤ 0.05, Supplementary Table S2). These findings support links to neurophysiological processes affected by childhood adversity.

Sequence motif enrichments to identify transcription factors binding sites among the 49 intervention-sensitive DMLs revealed 21 significantly enriched motifs (E-value < 0.05, Table 3). The top 5 sequence motifs corresponded to binding sites for ETV4, ZN341, ETV2, SP1, and BC11A transcription factors, which are involved...
| CpG ID   | Chromosome | Position | Strand | Relative to island position | FDR  | P value | Mean difference (t2−t1) | UCSC reference gene symbol | UCSC reference gene name   | UCSC reference gene group | UniProt function                                                                 |
|----------|------------|----------|--------|-----------------------------|------|--------|------------------------|--------------------------|-----------------------------|---------------------------|-----------------------------------------------------------------------------------|
| cg18252633 | chr11      | 73054401 | −      | S_Shore                     | 0.41 | 8.25E−07 | −0.10                  | ARHGGEF17                |                               |                           | Acts as guanine nucleotide exchange factor (GEF) for RhoA GTPases, involved in actine cytoskeleton organization. |
| cg1761483  | chr17      | 70723386 | −      | OpenSea                     | 0.65 | 5.85E−06 | 0.15                   | SLC39A11                  | Solute carrier family 39 member 11 | Body                       | Functions as a solute carrier family member.                                      |
| cg1377646  | chr1       | 11455041 | +      | OpenSea                     | 0.69 | 1.45E−05 | 0.11                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg00537196 | chr14      | 52688271 | −      | OpenSea                     | 0.49 | 2.67E−05 | 0.14                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg16270222 | chr17      | 41446396 | −      | Island                      | 0.65 | 5.03E−05 | 0.10                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg08827579 | chr9       | 11715048 | +      | OpenSea                     | 0.69 | 5.20E−05 | 0.15                   | AKNA                      | AT-Hook transcription factor | S’UTR                      | Centrosomal protein that plays a key role in cell delamination by regulating microtubule organization; involved in regulation of transcription and inflammatory responses |
| cg05105832 | chr10      | 64520254 | −      | OpenSea                     | 0.66 | 5.57E−05 | 0.13                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg21052873 | chr12      | 12493857 |  +    | N_Shelf                     | 0.76 | 7.37E−05 | 0.10                   | NCO2                      | Nuclear receptor corepressor 2 | Body                       | Functions as a transcriptional corepressor, involved in the regulation of several signaling pathways such as Notch |
| cg20497635 | chr17      | 998504   | +      | OpenSea                     | 0.76 | 1.14E−04 | 0.17                   | ABR                       | Active BCR-related gene     | Body                       | Functions as an important regulator of RAC1 activity in neurons and macrophages (regulating synaptic transmission, and GTPase mediated signal transduction) |
| cg02202133 | chr9       | 12631232 | −      | OpenSea                     | 0.76 | 1.20E−04 | −0.12                  | DENND1A                    | DENN domain containing 1A   | Body                       | Glycosyltransferase targetting Notch proteins and coagulation factors, among others |
| cg16306870 | chr3       | 19486897 | +      | OpenSea                     | 0.79 | 1.93E−04 | 0.18                   | XXYL1; CInsfp21            | Xyloside xylosyltransferase 1 | Body                       | Glycosyltransferase involved in cell adhesion, mediates interactions between cells and the extracellular matrix |
| cg03208742 | chr12      | 12447543 |  −    | OpenSea                     | 0.69 | 1.95E−04 | −0.10                  | ZNF6642;ZNF664; EAM10IA    | Zinc finger protein 664     | 5’UTR                      | Zinc finger protein involved in transcriptional regulation                        |
| cg25735425 | chr17      | 40307262 | −      | Island                      | 0.79 | 2.32E−04 | 0.11                   | RAB5C                      | Ras-related protein Rab-SC  | TSS1500                    | GTP-binding protein involved in protein transport and vesicular traffic          |
| cg17418085 | chr1       | 31229122 | −      | OpenSea                     | 0.79 | 2.36E−04 | 0.15                   | LAPT5                      | Lysosomal-associated transmembrane protein 5 | Body                       | Transmembrane receptor associated with lysosomes; involved in embryogenesis and in adult hematopoiesis |
| cg01569346 | chr6       | 32064148 |  +    | Island                      | 0.80 | 3.42E−04 | −0.19                  | TNXB                      | Tenascin-X                  | Body                       | Involved in cell adhesion, mediates interactions between cells and the extracellular matrix |
| cg07069368 | chr6       | 45294931 | −      | OpenSea                     | 0.65 | 3.44E−04 | 0.20                   | RUNX2; SUPT3H              | Runt-related transcription factor 2 | TSS1500;5’UTR; Body          | Transcription factor involved in osteoblastic differentiation and skeletal morphogenesis |
| cg05884705 | chr15      | 40600099 | +      | OpenSea                     | 0.79 | 3.52E−04 | 0.17                   | PCL2                       | Phospholipase C Beta 2       | 1stExon;5’UTR               | Phosphodiesterase involved in lipid metabolism and signal transduction            |
| cg21005774 | chr14      | 22917452 | +      | OpenSea                     | 0.79 | 3.78E−04 | 0.18                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg16002891 | chr12      | 6753017 | +      | N_Shelf                     | 0.80 | 4.23E−04 | 0.16                   | ACRBP                      | Acrosin-binding protein     | Body                       | Acrosomal protein involved in the acrosome formation                             |
| cg24365795 | chr16      | 28506015 | −      | N_Shelf                     | 0.79 | 4.45E−04 | 0.16                   | APO8R                      | Apolipoprotein B receptor   | 1stExon                    | Macrophage receptor involved in cholesterol and triglycerides metabolism, and lipid transport |
| cg10373891 | chr13      | 52338758 | +      | OpenSea                     | 0.79 | 4.88E−04 | 0.11                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg25946790 | chr14      | 90187489 | −      | OpenSea                     | 0.79 | 4.91E−04 | 0.12                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg01210113 | chr16      | 11352835 | −      | S_Shelf                     | 0.79 | 4.91E−04 | 0.11                   | −                         | −                           | −                         | −                                                                                                                                   |
| cg15210829 | chr17      | 2295425  | +      | N_Shore                     | 0.79 | 5.01E−04 | 0.17                   | MNT                       | Max-binding protein MNT     | Body                       | Binds DNA as a heterodimer with MAX and represses transcription                   |

Continued
| CpG ID | Chromosome | Position | Strand | Relative to island position | FDR | P value | Mean difference (t1−t2) | UCSC reference gene symbol | UCSC reference gene name | UCSC reference gene group | UniProt function |
|--------|------------|----------|--------|----------------------------|------|---------|----------------------|---------------------------|--------------------------|--------------------------|-------------------|
| cg14909856 | chr9 | 117150236 | + | OpenSea | 0.79 | 5.20E−04 | 0.19 | AKNA | Microtubule organization protein AKNA | 5'UTR | Body | Centrosomal protein that plays a key role in cell delamination by regulating microtubule organization; involved in regulation of transcription and inflammatory responses |
| cg16959766 | chr7 | 36230458 | + | OpenSea | 0.79 | 5.21E−04 | 0.10 | EEPD1 | Endonuclease/exonuclease/phosphatase family domain-containing protein 1 | Body | Body | Regulates gene expression linked to cholesterol transport and efflux |
| cg22461919 | chr16 | 71843295 | - | S_Shore | 0.76 | 5.30E−04 | 0.13 | AKNA | Microtubule organization protein AKNA | 5'UTR | TSS1500 | Subunit of clathrin-associated adaptor protein complex 1 that plays a role in protein sorting in the late-Golgi/trans-Golgi network (TGN) and/or endosomes |
| cg06536724 | chr17 | 64544418 | - | OpenSea | 0.79 | 5.31E−04 | 0.16 | PRKCA | Protein kinase C alpha type | Body | Body | Calcium-activated serine/threonine-protein kinase involved in apoptosis, cell adhesion, angiogenesis, platelet function and inflammation |
| cg18169886 | chr2 | 25517869 | + | OpenSea | 0.79 | 5.40E−04 | 0.13 | DNMT3A | DNA (cytosine-5)-methyltransferase 3A | Body | Body | Required for genome-wide de novo methylation and for the establishment of DNA methylation patterns during development |
| cg24498454 | chr19 | 48673965 | - | S_Shore | 0.79 | 5.43E−04 | 0.13 | LIG1,C19orf68 | Leucine-rich repeats and immunoglobulin-like domains protein 1 | TSS200;1stExon; 5'UTR | Body | Feedback negative regulator of signaling by receptor tyrosine kinases |
| cg19913426 | chr17 | 55213600 | - | OpenSea | 0.76 | 5.61E−04 | 0.17 | AKNA | Microtubule organization protein AKNA | 5'UTR | 5'UTR | |
| cg06066908 | chr6 | 138044052 | + | OpenSea | 0.79 | 5.90E−04 | 0.15 | AKNA | Microtubule organization protein AKNA | 5'UTR | 5'UTR | |
| cg18700133 | chr17 | 8013202 | - | Island | 0.77 | 6.02E−04 | −0.18 | ALOXE3 | Hydrolase: ceramide hydrolase; sphingolipid hydrolase | Body | Body | Lipoxigenase involved in lipid metabolism (hydroperoxy eicosatetraenoic acid biosynthesis and sphingolipid metabolism) |
| cg20055664 | chr8 | 134216562 | - | OpenSea | 0.79 | 6.37E−04 | −0.10 | WISP1 | WNT1-inducible signaling pathway protein 1 | Body | Body | Downstream regulator in the Wnt/Frizzled-signaling pathway, associated with cell survival |
| cg07922719 | chr9 | 117150338 | + | OpenSea | 0.78 | 6.55E−04 | 0.15 | AKNA | AT-Hook transcription factor | 5'UTR | 5'UTR | Centrosomal protein that plays a key role in cell delamination by regulating microtubule organization; involved in regulation of transcription and inflammatory responses |
| cg26091486 | chr20 | 2687292 | + | OpenSea | 0.80 | 6.55E−04 | 0.12 | EBF4 | EBF family member 4 | Body | Body | Transcriptional factor which recognizes variations of the palindromic sequence 5’-ATT CCCNNGGAAATT-3’ |
| cg26813601 | chr15 | 91105486 | + | OpenSea | 0.79 | 7.23E−04 | 0.18 | CRTC3 | CREB-regulated transcription coactivator 3 | Body | Body | Transcriptional coactivator for CREB1 involved in mitochondrial biogenesis, macrophage activation, lipid catabolism, etc |
| cg16815249 | chr6 | 111441357 | - | OpenSea | 0.79 | 7.46E−04 | 0.11 | SLC16A10 | Solute carrier family 16 member 10 | Body | Body | Sodium-independent transporter that mediates the uptake of aromatic acids (involved in thyroid hormone metabolism) |
| cg08609270 | chrX | 144903125 | + | Island | 0.76 | 7.94E−04 | −0.12 | SLITRK2 | SLIT and NTRK-like family member 2 | 1stExon; 5'UTR | 5'UTR | Protein involved in synaptic plasticity that promotes excitatory synapse differentiation |
| cg12078157 | chr6 | 13612218 | - | N_Shelf | 0.69 | 7.95E−04 | 0.13 | SIRT5 | Sirtuin 5 | 3'UTR | 3'UTR | Mitochondrial NAD-dependent deacetylase involved in mitochondrial organization, reactive oxygen species metabolism, etc |

Continued
| CPG ID   | Chromosome | Position | Strand | Relative to island position | FDR  | \( p \) value | Mean difference (T2–T1) | UCSC reference gene symbol | UCSC reference gene name | UCSC reference gene group | UniProt function                                                                 |
|---------|------------|----------|--------|-----------------------------|------|-------------|--------------------------|---------------------------|---------------------------|----------------------------|--------------------------------------------------------------------------------|
| cg26360755 | chr19  | 51539314 | +      | S_Shelf                      | 0.80 | 8.02E−04   | 0.10                     | KLLK12                    | Kalikrein-12               | TSS1500                   | Protein with peptidase activity                                     |
| cg11913565 | chr9   | 157814810 | -      | OpenSea                      | 0.80 | 8.07E−04   | 0.16                     | -                         | -                         | -                         | -                                                                        |
| cg05968174 | chrX  | 24187388 | -      | OpenSea                      | 0.78 | 8.61E−04   | 0.15                     | ZFX                       | X-chromosomal protein       | 5’UTR                     | Probable transcriptional activator                                  |
| cg21110034 | chr5   | 130752683 | +      | OpenSea                      | 0.79 | 8.71E−04   | 0.17                     | -                         | -                         | -                         | -                                                                        |
| cg25550677 | chr6   | 43027568 | +      | Island                       | 0.80 | 8.91E−04   | 0.14                     | KLC4;MRPL2                | Kinesin light chain 4      | TSS1500                   | Microtubule-associated force-producing protein that plays a role in organelle transport |
| cg22348534 | chr8   | 37887424 | +      | N_Shore                      | 0.79 | 9.10E−04   | 0.16                     | EIF4EBP1                  | EIF4EBP1 protein 1          | TSS1500                   | -                                                                        |
| cg13544012 | chr9   | 135709670 | -     | OpenSea                      | 0.80 | 9.10E−04   | 0.11                     | C9orf98                   | Adenylate kinase 8         | Body                      | Nucleoside monophosphate (NMP) kinase that catalyzes the reversible transfer of the terminal phosphate group between nucleoside triphosphates and monophosphates |
| cg13356427 | chr1   | 6520354  | +      | N_Shore                      | 0.79 | 9.26E−04   | 0.14                     | ESPN                     | ESPN                    | 3’UTR                     | Multifunctional actin-bundling protein                                |
| cg01515803 | chr13  | 51288187 | +      | OpenSea                      | 0.79 | 9.50E−04   | 0.15                     | DLEU7                     | Leukemia-associated protein 7 | Body                      | Protein coding gene deleted in Lymphoblastic Leukemia 7             |

Table 1. Intervention-sensitive differentially methylated loci (DML) with \( p \) value lower than 0.001 and a DNA mean difference (T2–T1) of 10% or more (n = 49). Function of the associated gene is reported as in UniProt database.

In cell differentiation, regulation of immune homeostasis, blood cell differentiation, immune responses, cancer, cardiovascular disease, diabetes and brain development, respectively, among other biological processes (UniProt database).

**Impact of multimodal intervention on epigenetic age acceleration.** Pearson's correlation analysis revealed no association between baseline Intrinsic Epigenetic Age Acceleration (IEAA) and ACE total score (n = 44; \( p \) value = 0.43; \( r = -0.13 \)). The analyses of the three categories of adversity assessed by the standard ACE questionnaire (i.e. abuse, neglect and household challenges), revealed a weak but significant positive correlation between IEAA and exposure to abuse (emotional, physical and sexual) (\( p \) value = 0.03; \( r = 0.33 \)) while neglect (emotional and physical) and household challenges (separation from biological parents, witnessing domestic violence, household substance abuse, mental illness in household and having incarcerated family members) were not associated with epigenetic accelerated aging (neglect: \( p \) value = 0.07; \( r = 0.27 \); household challenges: \( p \) value = 0.13; \( r = -0.23 \)). No significant difference was found in DNA methylation age or Intrinsic Epigenetic Age Acceleration (IEAA) between groups, calculated at T1 and T2 (Fig. 2; Supplementary Table S3a). The intervention did not have any significant impact on the participants' IEAA according to the ANCOVA model (coefficient = −0.661, SE = 0.874, \( p \) value = 0.454) (Supplementary Table S3b).

**Correlation between psychological and DNA methylation outcomes.** Since we previously reported a significant improvement in attention/awareness-related outcomes and a reduction in trauma-related outcomes following the 1-week intervention group program\(^a\), we next sought to identify DNA methylation changes related to psychological outcomes by comparing differences in DNA methylation levels and changes in the scores for Attention Awareness Scale-Adolescents (MAAS-A), trauma (the Short PTSD Rating Interview (SPRINT)), and the Child PTSD Symptom Scale (CPSS) at baseline (T1) and post-intervention (T2). This approach revealed significant correlations of DNA methylation levels at 274 CpGs with MAAS-A scores (\( p \) value < 1 \times 10^{-5}; \( r > 0.5 \); Supplementary Table S4). However, none of these CpGs corresponded to the intervention-sensitive DML described above and they did not show significant functional enrichment (Supplementary Table S5). Improved SPRINT and CPSS scores significantly correlated with DNA methylation levels at 160 and 202 CpGs, respectively (\( p \) value < 1 \times 10^{-3}; \( r > 0.5 \); Supplementary Tables S6 and S7). Two of these genes corresponded to the intervention-sensitive DMLs described above: SIRT5 gene (Sirtuin 5; \( p \) value: 0.0001, \( r = -0.59 \)) and TRAPPCL2 gene (Trafficking Protein Particle Complex Subunit 2L; \( p \) value: 0.00002, \( r = -0.55 \); Supplementary Table S1). The DNA methylation levels at 35 CpGs correlated with both CPSS and SPRINT scores and Fisher test confirmed that the CpG overlap between scales was significant (\( p \) value < 1 \times 10^{-5}). This observation...
is consistent with the fact that both SPRINT and CPSS scales measure PTSD-related outcomes and that the results from both scales were highly correlated in our previous report \((r = 0.833, p < 1 \times 10^{-4})\). Annotation of these 35 CpGs to genes revealed the known functions of the encoded proteins (Table 4) and an enrichment analysis detected functional interactions involved in metabolic, cardiovascular, immune and neural signaling \((q\text{-value} < 0.04, \text{Supplementary Table S8})\).

**Discussion**

Here we describe a genome-wide DNA methylation analysis from saliva samples, as an extension of our previous study that showed the mental health benefits of an intensive multimodal 1-week group program involving mindfulness training, artistic expression and EMDR in adolescent girls with a history of 4 or more ACEs (full details on the program protocol and psychological outcomes are described in Roque Lopez et al.\(^{36}\)).

Forty-nine DML were sensitive to the intervention with a methylation change greater than 10% \((p\text{-value} < 0.001)\). Fifty-four percent of these DML were located in the body of genes, of which 76% showed increases in DNA methylation levels post-intervention, which is generally associated with active transcription in proliferative tissues\(^{37}\).

Although DNA methylation analysis from saliva samples might be not representative of other tissue type programming, some reports have shown correlations between DNA methylation levels in brain, blood and saliva\(^{38-41}\). A biological pathway-enrichment analysis of the 49 intervention-sensitive DML-associated genes suggests the modulation of several functional processes associated with diseases linked to early childhood adversity, including several biological processes involved in neural signaling and substance abuse disorders (e.g., glutamate receptor, beta agonist/beta blocker, cholinergic, glutamatergic, serotonergic and dopaminergic synapses and opioid, oxytocin and endocannabinoid signaling, long-term depression and potentiation). These findings are consistent with other reports showing that ACEs can trigger DNA methylation changes in genes that modulate mental health and behavior, such as serotonin transporter and glucocorticoid receptor genes\(^{42-44}\), brain-derived neurotrophic factor\(^{45}\) and glutamate receptor\(^{46}\), oxytocin receptor\(^{47-51}\). DML-associated genes also were enriched in processes involved in neural signaling and substance abuse disorders (e.g., glutamate receptor, beta agonist/ beta blocker, cholinergic, glutamatergic, serotonergic and dopaminergic synapses and opioid, oxytocin and endocannabinoid signaling, long-term depression and potentiation). In addition, these DML-associated genes were significantly enriched in processes involved in cardiovascular health (e.g., endothelins, vascular smooth muscle contraction, thromboxane A2 receptor and calcium signaling, beta-agonist/beta-blocker pathways), diabetes (e.g., insulin secretion, leptin signaling, pancreatic secretion, AGE-RAGE signaling) and cancer (e.g., choline metabolism, WNT, ErbB and EGF-EGF receptor signaling, cancer-related microRNAs, NOTCH signaling), which are non-communicable diseases more likely to appear in 18 year old adults or older with a history of at least 4 ACEs than in those with none\(^1\). Inflammation also has been reported in stress-related disorders\(^{42,43}\) and the enrichment analysis suggests that the intervention may regulate inflammation through the modulation of IL8- and chemokine G-coupled receptor CXCRI-1 and CXCRI2-mediated signaling. Furthermore, stress-related DNA methylation changes were associated with the enrichment in several hormone networks (e.g., follicle stimulating hormone signaling, thyroid hormone synthesis and signaling, androgen receptor signaling, aldosterone synthesis and secretion), which are regulated by hypothalamic-pituitary endocrine axes known to be sensitive to stress and childhood adversity\(^{44,45}\). Consistent with these findings, the top 5 significantly enriched DNA sequence motifs corresponding to transcription factors binding sites are involved in the regulation of similar processes. ETV4 and ETV2 are transcription factors of the ETS family that have been largely involved in carcinogenesis\(^{46}\) and cardiovascular disease\(^{47}\). Specificity protein 1 (SP1) is associated with different types of cancer, neurological and cardiovascular disease\(^{48-50}\) and ZNF341 is involved in immune-mediated disorders and infection susceptibility by regulating IL-6 signaling\(^{51}\). BCL11A is involved in β-hemoglobinopathies, cancer and type II diabetes\(^{52}\), neurogenesis\(^{49}\) and midbrain dopaminergic neurons\(^{53}\).

In our study we found no evidence of association between IEAA and ACE total score, probably because 90% of the participants had a history of 4 or more ACEs. However, our analyses of the three categories of adversity (i.e. abuse, neglect and household challenges), revealed a weak but significant correlation between IEAA and exposure to abuse (emotional, physical and sexual) but not to the other ACE categories. These findings are consistent with data from a prospective study with 974 children showing that girls from age 0–14 years exposed to abuse (emotional, physical and sexual) but not to the other ACE categories, revealed a weak but significant correlation between IEAA and psychological outcomes at 202, 160, and 274 CpGs, respectively. However, only two of these DML, annotated to the SIRT5 and TRAPPC2L genes, showed a change in DNA methylation level greater than 5% \((p\text{-value} < 0.001)\). SIRT5 (change in DNA methylation = 13%) was associated with CPSS scores and TRAPPC2L (change in DNA methylation = 7%) was associated with SPRINT scores. SIRT5 is a member of the sirtuin family of proteins located predominantly in the mitochondrial matrix, and it protects cells from oxidative stress\(^{54,55}\). The effect of traumatic stress on oxidative components and redox-state homeostasis has been documented\(^59\). These data suggest that the epigenetic modulation of antioxidant-related pathways may be relevant to the psychological benefits of the intervention. SPRINT scores negatively correlated with the DNA methylation levels at the body of TRAPPC2L gene, which is involved in intracellular vesicle-mediated transport events\(^{56}\) and is functionally associated with neurodevelopmental delay/intellectual disabilities in individuals homozygous for a missense variant\(^{57}\).
Taken together, our data support the contribution of epigenetic mechanisms in mediating the effects of the 1-week intervention group program for adolescents exposed to 4 or more ACEs. Future studies are required to examine the functional implications of these changes (i.e., expression levels and activity of candidate genes). The potential relationships of these findings with physiological outcomes may help identify molecular targets aimed to prevent the onset of health disorders and improve the long-term health trajectory in individuals with 4 or more ACEs. Although this level of exposure to adversity increases the risk of adult onset of chronic health problems, behavioral risk, and mortality3, ACE screening is not yet integrated into primary care. One of the arguments is the scarce evidence on therapeutic strategies for children or adolescents with a history of multiple victimization62. However, the early screening of ACEs is seen by several authors as a promising way to promote child well-being through policy, health education and evidence-based programs for families, children and adolescents63,64. Results presented in our previous study36, data presented here and recent evidence from other studies31,32,65,66 are starting to provide the scientific background to encourage further discussions on future avenues for prevention and treatment of ACEs. Although this study describes a promising short intervention for adolescents with multiple ACEs, the participants may still need group or individual follow-up support in order to enhance and strengthen the benefits from this program. Future prospective studies to assess the stability of the epigenetic changes resulting from the intervention and their potential long-term influence on health are warranted.

**Methods**

**Participants.** We recruited forty-four adolescent girls, aged 13–16 years, from the foster care system of the Colombian Institute of Family Well-Being (ICBF). All participants were partially or totally separated from their biological families due to inadequate parental care, including abuse and neglect. Exclusion criteria were cognitive impairment, self-harming behavior within the last 6 months, suicidal behavior or ideation or current...
A hot beverage in the garden, followed by a 30 min yoga session and a guided loving kindness and compassion meditation to cultivate positive affective states. After a healthy breakfast, participants attended a mindfulness practice for adolescents. The program included several sessions per day of artistic expression through art and craft, dramatic play, dance, and music. On days 5 and 6, participants attended two EMDR group protocol sessions/day. During that same week, the control group was engaged in holiday activities proposed by the ICBF.

No samples showed incorrect sex prediction based on methylation levels. Probes were filtered if at most one value exceeded 0.05. Probes were normal-exponential out-of-band (noob) normalized with dye correction, followed by quantile normalization. No samples showed incorrect sex prediction based on methylation levels. Probes were filtered if at most one value < 0.001 and mean difference (T2−T1) > 10% using the ConsensusPath tool.

| Consensus Path name | Functional set id | p value | FDR q-value |
|---------------------|-------------------|---------|-------------|
| Acetylcholine regulates insulin secretion (Reactome) | 118332 | 0.0002 | 0.023 |
| Hematopoietic stem cell gene regulation by GABF alpha-beta complex (Wikipathways) | 3874547 | 0.0007 | 0.023 |
| Anoxia—Homo sapiens (human) (KEGG) | 167455 | 0.0010 | 0.023 |
| Regulation of sfrf-4e and p70s6 kinase (BioCarta) | 282015 | 0.0012 | 0.023 |
| Parathyroid hormone synthesis secretion and action—Homo sapiens (human) (KEGG) | 167307 | 0.0013 | 0.023 |
| Follicle Stimulating Hormone (FSH) signaling pathway (Wikipathways) | 3874074 | 0.0014 | 0.023 |
| IL-8- and CXCR1-mediated signaling events (PID) | 264396 | 0.0015 | 0.023 |
| GPCR Group1 metabotropic glutamate receptor (INOH) | 299561 | 0.0015 | 0.023 |
| Thyroid hormone signaling pathway—Homo sapiens (human) (KEGG) | 167527 | 0.0017 | 0.023 |
| Retinoic acid receptors-mediated signaling (PID) | 264415 | 0.0017 | 0.023 |
| Alpha 6 Beta 4 signaling pathway (Wikipathways) | 3874238 | 0.0021 | 0.024 |
| IL-8- and CXCR2-mediated signaling events (PID) | 264520 | 0.0022 | 0.024 |
| African trypanosomiasis—Homo sapiens (human) (KEGG) | 167452 | 0.0024 | 0.024 |
| Target Of Rapamycin (TOR) signaling (Wikipathways) | 3873991 | 0.0025 | 0.024 |
| PAR1-mediated thrombin signaling events (PID) | 264368 | 0.0037 | 0.028 |
| PLC beta mediated events (Reactome) | 46932 | 0.0037 | 0.028 |
| G-protein mediated events (Reactome) | 46967 | 0.0039 | 0.028 |
| Proton pump inhibitor pathway pharmacodynamics (PharmGKB) | 3193117 | 0.0040 | 0.028 |
| Endocrine and other factor-regulated calcium reabsorption—Homo sapiens (human) (KEGG) | 167435 | 0.0042 | 0.028 |
| Regulation of RhoA activity (PID) | 264551 | 0.0044 | 0.028 |

Table 2. Top 20 functional interactions of the 49 meditation-sensitive DML (p value < 0.001 and mean difference (T2−T1) > 10%) using the ConsensusPath tool.

Intervention. The intervention was performed during a school holiday week (June 20–27th, 2019) and it was conducted at a nature retreat facility in Santander, Colombia. The intervention program included an early morning routine starting with an awakening with soft music and a hot beverage in the garden, followed by a 30 min yoga session and a guided loving kindness and compassion meditation to cultivate positive affective states. After a healthy breakfast, participants attended a mindfulness practice for adolescents. The program included several sessions per day of artistic expression through art and craft, dramatic play, dance, and music. On days 5 and 6, participants attended two EMDR group protocol sessions/day. During that same week, the control group was engaged in holiday activities proposed by the ICBF. While the intervention and the control group, in their respective locations, engaged in some similar activities (e.g., dance, acting, physical exercise, games, movies), the control group activities did not include approaches to specifically treat traumatic experiences or to promote attentional and emotional regulation. For full details of the intervention program and the control group activities, see Roque López et al.36.

DNA isolation and Methylation microarray. Before and after the 1-week intervention, saliva samples (1 ml) from all the participants (n = 22/group) were collected using Oragene saliva collection kits and DNA was isolated according to the manufacturer’s protocol. DNA concentration was determined using a Qubit fluorometer (Life Technologies) and normalized to 20 ng/μl for the methylation microarray. Bisulfite conversion was performed with the EZ methylation Gold-kit (cat# D5005, Zymo Research) and the Illumina Infinium MethylationEPIC Beadchip Array was used to quantitatively interrogate at single-nucleotide resolution over 850,000 CpG sites across the genome (Biotech Center, University of Wisconsin-Madison).

Pre-processing of human MethylationEPIC data. Raw intensity data files were imported into R environment. R package minfi was used to assess sample quality, calculate the detection p value of each tested probe, and normalize data. Two samples were discarded as their mean detection p value exceeded 0.05. Probes were normal-exponential out-of-band (noob) normalized with dye correction, followed by quantile normalization. No samples showed incorrect sex prediction based on methylation levels. Probes were filtered if at most one...
| Rank | Transcription factor | Adj. P value (FDR) | E value | No of DML | UCSC reference gene name | UniProt function |
|------|----------------------|-------------------|---------|-----------|--------------------------|------------------|
| 1    | ETV4                 | 1.56E−08          | 6.24E−06| 22        | ETS translocation variant 4 | Transcriptional activator involved in cell differentiation |
| 2    | ZN341                | 2.10E−07          | 8.41E−05| 21        | Zinc finger protein 341   | Transcriptional activator of STAT3 involved in the regulation of immune homeostasis |
| 3    | ETV2                 | 3.85E−07          | 1.54E−04| 26        | ETS translocation variant 2 | Transcriptional activator involved in blood cells differentiation, Notch and Wnt signalling pathways |
| 4    | SP1                  | 1.63E−06          | 6.53E−04| 8         | Transcription factor Sp1   | Transcriptional factor that regulates the expression of genes involved in cell growth, apoptosis, angiogenesis, differentiation and immune responses |
| 5    | BC11A                | 2.48E−06          | 9.96E−04| 25        | B-cell lymphoma/leukemia 11A | Transcription factor involved in brain development, hematopoiesis, lymphopoiesis |
| 6    | ERG                  | 2.97E−06          | 1.19E−03| 25        | ETS Transcription Factor ERG | Transcriptional regulator in cell differentiation |
| 7    | SPI1                 | 3.39E−06          | 1.36E−03| 16        | Transcription factor PU.1   | Transcriptional activator involved in the differentiation or activation of macrophages or B-cells |
| 8    | IRF2                 | 5.87E−06          | 2.35E−03| 12        | Interferon regulatory factor 2 | Transcriptional activator involved in immune response |
| 9    | ELF3                 | 7.34E−06          | 2.94E−03| 22        | ETS-related transcription factor Elf-3 | Transcriptional factor involved in cell differentiation, extracellular matrix organization and inflammatory response |
| 10   | SP3                  | 8.08E−06          | 3.24E−03| 16        | Transcription factor Sp3   | Transcriptional activator of genes involved in cell-cycle regulation, hormone-induction and house-keeping |
| 11   | ETV5                 | 8.14E−06          | 3.27E−03| 37        | ETS translocation variant 5 | Transcription factor involved in cell differentiation and cellular response to oxidative stress |
| 12   | IRF8                 | 1.45E−05          | 5.83E−03| 19        | Interferon regulatory factor 8 | Transcription negative regulator in cells of the immune system, involved in the immune response |
| 13   | KLF15                | 1.48E−05          | 5.94E−03| 15        | Krueppel-like factor 15    | Transcription factor involved in many processes such as glucose homeostasis, insulin response, Wnt signalling pathway |
| 14   | VEZF1                | 2.69E−05          | 1.08E−02| 23        | Vascular endothelial zinc finger 1 | Atypical E2F transcription factor that participates in various processes such as angiogenesis, polyplidization of specialized cells and DNA damage response |
| 15   | E2F7                 | 3.69E−05          | 1.48E−02| 17        | Transcription factor E2F7   | Transcription factor involved in cellular defense response and angiogenesis |
| 16   | SP4                  | 5.12E−05          | 2.05E−02| 20        | Transcription factor Sp4    | Transcriptional activator |
| 17   | SPIB                 | 5.86E−05          | 2.35E−02| 11        | Transcription factor Spi-B  | Transcriptional activator involved in cell differentiation that can act as a lymphoid-specific enhancer |
| 18   | IRF1                 | 6.05E−05          | 2.43E−02| 10        | Interferon regulatory factor 1 | Transcriptional regulator involved in immune response and apoptosis |
| 19   | EHF                  | 7.73E−05          | 3.10E−02| 22        | ETS homologous factor      | Transcriptional activator involved in regulating epithelial cell differentiation and proliferation |
| 20   | ZN770                | 8.21E−05          | 3.29E−02| 21        | Zinc finger protein 770    | Transcription regulator |
| 21   | ELF5                 | 1.04E−04          | 4.16E−02| 21        | ETS-related transcription factor Elf-5 | Transcriptionally activator involved in cell differentiation, that regulates the later stages of keratinocytes terminal differentiation. |

Table 3. Transcription factor motif enrichment analysis of intervention-sensitive DML. DNA sequences flanking the 49 intervention-sensitive DML (+/− 250 bp) were used to identify enriched motifs using the AME suite package (p value ≤ 0.0001; E-value ≤ 0.05). Transcription factors predicted to bind to each motif, Bonferroni adjusted p value, E-value, and the number of DML where the motif is present are shown. Functions of the transcription factors were obtained using the UniProt database.

Sample exhibited a detection p value > 0.01, contained a SNP, reported methylation at a SNP, measured methylation at a CH dinucleotide site, had at most one sample with a detection p value > 0.01 or were known cross-reactive probes. These filtration criteria resulted in 688,000 probes used for further analysis.

Beta values were obtained through minfi and were further converted to M-values for differential analysis.

**Identification of differentially methylated loci (DML).** Linear regression for each tested CpG using an ANCOVA model was employed using R package limma. The treatment effect (difference between the intervention and control group) on DNA methylation level, was estimated using an analysis of covariance (ANCOVA) of the outcome (T2) with the baseline (T1) as covariate. BMI, age, ACE score and cell type proportions (surrogate variables) were also included as covariates. In this model, the mean posttest difference between the groups is the outcome (T2) with the baseline (T1) as covariate. BMI, age, ACE score and cell type proportions (surrogate variables) were also included as covariates. In this model, the mean posttest difference between the groups is

...
array (measure of continuous variables in large cell numbers, non-variability of many sites on the array, correlation between neighboring probes on the array) likely resulted in the absence of FDR adjusted DML. However, an FDR adjustment assumes independence in the comparisons, and DNA methylation levels across the genome are not independent. Thus, several studies have taken an approach that requires a larger effect size (i.e., > 10%) with a more liberal \( p \) value cut-off. Therefore, to detect intervention-sensitive DML, we established as cut-off a \( p \) value ≤ 0.001 combined with an average difference in methylation between T1 and T2 greater than 10%.

**Functional analysis.** Gene ontological enrichment of biological processes were identified using the ConsensusPathDB-human database as implemented in the Functional Enrichment module of the EASIER R package. This database integrates interaction networks in Homo sapiens including metabolic, biochemical and gene regulatory signaling and drug-target interactions. FDR-corrected \( p \) values < 0.05 were considered significant.

The DNA sequences flanking the DML of (+ /− 250 nucleotides) were used to find enriched motifs using the AME suite package (MEME Suite online platform). An E-value cut-off of 0.05 was established to identify significantly enriched motifs, as recommended by MEME developers.

**Estimation of the impact of the multimodal intervention on epigenetic age acceleration.** We explored the associations between Intrinsic Epigenetic Age Acceleration (IEAA) and ACE scores using the basal DNA methylation data from both groups and the ACE scores that we previously described in the same sample. Child epigenetic age based on the Pediatric-Buccal-Epigenetics’ (PedBE) clock was calculated using the methylation clock R package. The package provides the following parameters: (i) DNA methylation predicted age (biological age) in years, (ii) age acceleration, difference between DNAm and chronological age in years; (iii) Intrinsic Epigenetic Age Acceleration (IEAA), obtained after regressing chronological age and cell type proportions on biological age. Pearson's correlation analysis was used to explore associations between basal Intrinsic Epigenetic Age Acceleration (IEAA) and ACE total score and the number of ACEs from each one of the three categories of adversity (i.e. abuse: emotional, physical and sexual; neglect: emotional and physical; household dysfunction: separation from biological parents, witnessing domestic violence, household substance abuse, mental illness in household and having incarcerated family members), assessed by the 10-item ACE questionnaire derived from the Kaiser Permanente ACEs Study (full details on frequency and patterns of ACEs in this sample are described in Roque Lopez et al.). We used an ANCOVA model (see “Methods”) to assess the potential impact of the intervention on IEAA. This model included group (intervention or control) as the independent variable, IEAA at T2 from both groups as the dependent variable, and it was adjusted by basal IEAA (T1), BMI, and ACE score, considering \( p \) values < 0.05 as significant.

**Correlation between psychological phenotypic measures and DNA methylation.** PTSD and awareness and attention-related outcomes of this intervention in this same sample were assessed by SPRINT, CPSS and MAAS-A scales and are fully reported in our previous report. Here we conducted correlations between changes in the above-mentioned scales and changes in DNA methylation (T2−T1) of each CpG.
| CpG ID     | Chromosome | Position | Strand | Relative to island position | UCSC reference gene symbol | UCSC reference gene group | UCSC reference gene name | UniProt function                                                                 |
|------------|------------|----------|--------|-----------------------------|----------------------------|--------------------------|--------------------------|----------------------------------------------------------------------------------|
| cg11029504 | chr9       | 80512104 | +      | OpenSea                     | GNAQ                       | Body                     | Guanine nucleotide-binding protein G(q) subunit alpha | Guanine nucleotide-binding protein involved in transmembrane signaling systems, action potential, glutamate signaling pathway, and other processes, as modulator or transducer |
| cg19041132 | chr17      | 74380824 | -      | Island                      | SPHK1                      | 5’UTR; 1stExon; TSS1500  | Sphingosine kinase 1           | Protein kinase that catalyzes the phosphorylation of sphingosine to form sphingosine 1-phosphate, involved in the regulation of inflammatory response and neuroinflammation |
| cg07300846 | chr16      | 29888571 | +      | S_Shore                      | SEZ6L2                     | Body                     | Seizure 6-like protein 2     | Protein that contributes to specialized endoplasmic reticulum functions in neurons |
| cg22531801 | chr1       | 235806070 | +      | S_Shore                      | GNG4                       | 5’UTR                    | Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-4 | Guanine nucleotide-binding protein involved in transmembrane signaling systems, severla neurotransmitter signaling pathways, and other processes, as modulator or transducer |
| cg10595547 | chr10      | 119310911 | +      | N_Shore                      | –                          | –                        | –                        | Long non-coding RNA identified as a candidate oncogene                             |
| cg11478273 | chr8       | 128806682 | –      | Island                      | PVT1                       | TSS200                   | PVT1 Oncogene               | component With Sequence Similarity 155 Member B                                 |
| cg01759889 | chrX       | 68725086 | +      | Island                      | FAM155B                    | 1stExon; 5’UTR            | Family With Sequence Similarity 155 Member B | Component of the NALCN channel complex, involved in the regulation of the resting membrane potential and neuronal excitability |
| cg25902682 | chr5       | 79461463 | +      | OpenSea                      | SERINC5                    | Body                     | Serine incorporator 5         | Enhances the incorporation of serine into phosphatidylserine and sphingolipids, involved in immunity, lipid metabolism and myelin formation |
| cg05374956 | chr19      | 5838735 | –      | OpenSea                     | FUT6                       | 1stExon; 5’UTR            | 4-galactosyl-N-acetylglucosaminide 3-alpha-L-fucosyltransferase FUT6 | Glycosyltransferase protein involved in glycosylation and lipid metabolism |
| cg00672930 | chr8       | 130585711 | +   | OpenSea                      | CCDC26                     | Body                     | Putative coiled-coil domain-containing 26 | Long non-coding RNA identified in myelocyte-monocyte lineage |
| cg12153422 | chr14      | 75075712 | +      | N_Shore                      | LTPB2                      | Body                     | Latent-transforming growth factor beta-binding protein 2 | Plays an integral structural role in elastic-fiber architectural organization and/ or assembly |
| cg05694971 | chr15      | 36872036 | –      | S_Shore                      | C15orf41                   | 5’UTR, 1stExon            | CDAN1-interacting nuclease 1 | Involved in erythroid cell differentiation |
| cg20412539 | chr7       | 999153 | –      | OpenSea                      | –                          | –                        | –                        | –                                                                                 |
| cg17290488 | chr5       | 179281560 | –   | N_Shelf                      | C5orf45                    | Body                     | MRN complex-interacting protein | Involved in cellular response to DNA damage and the maintenance of genome stability through its association with the MRN damage-sensing complex |
| cg13451093 | chr9       | 137040612 | –      | OpenSea                      | –                          | –                        | –                        | –                                                                                 |
| cg15757326 | chr18      | 61704584 | +      | OpenSea                      | –                          | –                        | –                        | –                                                                                 |
| cg11856215 | chr11      | 63535358 | –      | N_Shore                      | C11orf95                   | Body                     | Zinc finger translocation-associated protein | Negative regulator of transcription |

Continued
| CpG ID     | Chromosome | Position | Strand | Relative to island position | UCSC reference gene symbol | UCSC reference gene group | UCSC reference gene name | UniProt function                                                                 |
|-----------|------------|----------|--------|-----------------------------|---------------------------|--------------------------|-------------------------|---------------------------------------------------------------------------------|
| cg19816811 | chr7       | 27188364 | +      | N_Shore                     | HOXA6                     | TSS1500                  | Homeobox protein           | Hox A6                                                                         |
|           |            |          |        |                             |                           |                          |                        | Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis |
| cg00094518 | chr7       | 130418549 | +      | Island                      | KLF14                     | 1stExon                  | Krueppel-like factor 14    |                                                                                   |
|           |            |          |        |                             |                           |                          |                        | Transcription factor involved in various processes including sphingolipid mediated signaling pathway |
| cg27380803 | chr17      | 62034801 | +      | OpenSea                     | SCN4A                     | Body                     | Sodium channel protein     | Type 4 subunit alpha                                                            |
|           |            |          |        |                             |                           |                          |                        | Subunit of a voltage-gated sodium channel complex, involved in neuronal action potential, muscle contraction, etc |
| cg22246918 | chr13      | 51094655 | -      | OpenSea                     | –                         | –                        | –                       | –                                                                               |
| cg11336382 | chr1       | 228658466 | -      | N_Shore                     | –                         | –                        | –                       | –                                                                               |
| cg11283677 | chr17      | 60727886 | -      | N_Shore                     | MRC2                      | Body                     | C-type mannose receptor 2  |                                                                                   |
|           |            |          |        |                             |                           |                          |                        | May play a role as endocytotic lectin receptor displaying calcium-dependent lectin activity; involved in collagen catabolism, endocytosis, etc |
| cg07401516 | chr5       | 95571107 | +      | OpenSea                     | –                         | –                        | –                       | –                                                                               |
| cg24513433 | chr18      | 47088234 | -      | Island                      | LIPG                      | TSS200                   | Endothelial lipase         |                                                                                   |
|           |            |          |        |                             |                           |                          |                        | Exerts both phospholipase and triglyceride lipase activities; involved in lipid metabolism and cell proliferation |
| cg20766178 | chrX       | 71131060 | +      | Island                      | NHSL2                     | 1stExon                  | NHS-like protein 2        |                                                                                   |
|           |            |          |        |                             |                           |                          |                        | Protein involved in cell differentiation                                         |
| cg07724623 | chr1       | 115397409 | -     | N_Shore                     | SYCP1                     | TSS200                   | Synaptomnal complex protein 1 |                                                                                   |
|           |            |          |        |                             |                           |                          |                        | Major component of the transverse filaments of synaptomnal complexes; involved in cell division |
| cg22154449 | chr18      | 56930452 | +      | N_Shore                     | –                         | –                        | –                       | –                                                                               |
| cg16941643 | chr9       | 127277206 | -     | OpenSea                     | –                         | –                        | –                       | –                                                                               |
| cg23341182 | chr10      | 102046768 | +     | S_Shore                     | BLOC1S2                   | TSS1500                  | Biogenesis of lysosome-related organelles complex 1 subunit 2 | Component of the BLOC-1 complex, involved in biogenesis of lysosome-related organelles, axonal transport, neurite extension, neuron differentiation, and other processes |
| cg20655103 | chr8       | 143792280 | -     | OpenSea                     | LOC100288181              | Body                     | LncRNA Associated With Ovarian Cancer 1 | Long non-coding RNA associated with ovarian cancer |
| cg19852286 | chr5       | 173237320 | +     | OpenSea                     | –                         | –                        | –                       | –                                                                               |
| cg06179698 | chr2       | 176671985 | -     | OpenSea                     | –                         | –                        | –                       | –                                                                               |
| cg06326092 | chr16      | 30034487 | -     | S_Shore                     | CL6or92                   | TSS200                   | Fertilization-influencing membrane protein | May play a role in sperm-oocyte fusion during fertilization |
| cg00454932 | chr1       | 171750547 | +     | Island                      | METTL13                   | TSS1500                  | eEF1A lysine and N-terminal methyl-transferase | Methyltransferase involved in the negative regulation of mRNA translation |

**Table 4.** Function (Uniprot database) of the genes associated to the 35 CpGs found to correlate with both CPSS and SPRINT scales.
Linear regression for each tested CpG using an ANCOVA model was employed using R package limma\textsuperscript{73}. Separate models for each psychological scale were constructed, controlling for age, BMI and ACEs score. Surrogate variables were assessed by the R package sva\textsuperscript{72}. To assess systematic bias of the linear regression model, the genomic inflation factor was calculated for the obtained p values, yielding a genomic inflation factor of ~1, suggesting no bias. Pearson's correlation coefficients (r) were calculated for continuous variables of interest with beta-values. Correlations with an uncorrected p value < 1 \times 10^{-3}, and a correlation coefficient r > 0.5 were considered significant for the current study.

Data availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Competing interests

The program described in this study was designed by author Susana Roque-López, founder and director of the nonprofit organization Association Innocence in Danger Colombia (IIDC).

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Author contributions

PK; E.LL.-A.; S.R.L.: Concept, experimental design and data acquisition; S.R.-L.: multimodal program design; PK, M.C.-T., A.M., L.P., R.S.A., R.J.D.: data analysis/interpretation; PK., M.C.-T.: drafting of the manuscript; R.S.A., R.J.D: critical revision of the manuscript; R.J.D, P.K., E.LL.-A., S.R.-L.: funding acquisition. All authors read and approved the final manuscript.

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Additional information

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