Neuropsychopharmacology www.nature.com/npp

ARTICLE

Intergenerational trauma is associated with expression alterations in glucocorticoid- and immune-related genes

Nikolaos P. Daskalakis[1,2,3], Changxin Xu[3,4], Heather N. Bader[3,4], Chris Chatzinakos[1,2], Peter Weber[5], Iouri Makotkine[3,4], Amy Lehrner[3,4], Linda M. Bierer[3,4], Elisabeth B. Binder[5,6] and Rachel Yehuda[3,4]

Offspring of trauma survivors are more likely to develop PTSD, mood, and anxiety disorders and demonstrate endocrine and molecular alterations compared to controls. This study reports the association between parental Holocaust exposure and genome-wide gene expression in peripheral blood mononuclear cells (PBMC) from 77 Holocaust survivor offspring and 15 comparison subjects. Forty-two differentially expressed genes (DEGs) were identified in association with parental Holocaust exposure (FDR-adjusted $p < 0.05$); most of these genes were downregulated and co-expressed in a gene network related to immune cell functions. When both parental Holocaust exposure and maternal age at Holocaust exposure shared DEGs, fold changes were in the opposite direction. Similarly, fold changes of shared DEGs associated with maternal PTSD and paternal PTSD were in opposite directions, while fold changes of shared DEGs associated with both maternal and paternal Holocaust exposure or associated with both maternal and paternal age at Holocaust exposure were in the same direction. Moreover, the DEGs associated with parental Holocaust exposure were enriched for glucocorticoid-regulated genes and immune pathways with some of these genes mediating the effects of parental Holocaust exposure on C-reactive protein. The top gene across all analyses was *MMP8*, encoding the matrix metalloproteinase 8, which is a regulator of innate immunity. To conclude, this study identified a set of glucocorticoid and immune-related genes in association with parental Holocaust exposure with differential effects based on parental exposure-related factors.

Neuropsychopharmacology (2021) 46:763–773; https://doi.org/10.1038/s41386-020-00900-8

INTRODUCTION

Parental traumatic exposures can affect physical and mental health of offspring [1–4]. Adult offspring of Holocaust survivors were shown to have more symptoms of anxiety, depression and PTSD than demographically-similar offspring of Jewish parents who were not exposed to the Holocaust [2, 5]. Similar associations were described in studies of Cambodian [3] and Rwandan [4] genocides. Studies on candidate biological systems have revealed alterations in basal cortisol excretion [6], pituitary and leukocyte glucocorticoid (GC) sensitivity [7], and GC metabolism [8] of offspring, many of which are similar to those associated with trauma survivors with PTSD, mood and anxiety disorders [9]. These studies have underscored the importance of parental symptoms, sex, and age at exposure, as well as offspring sex, experiences and symptoms as potential contributors to the presence and direction of dysregulated biological findings observed in offspring [1].

Studies examining the putative molecular basis of the strong GC-related signals observed in association with parental trauma exposure, have focused on epigenetic alterations of stress related genes in blood. Differences in DNA methylation (DNAm) in an intronic region of the FK506 binding protein 51 gene (*FKBP5*) have been associated with parental Holocaust exposure [10] and maternal age at Holocaust exposure [11], while DNAm changes in a promoter of the GC receptor (GR) gene were in opposite directions for offspring with maternal vs. paternal PTSD [12]. These epigenetic studies in targeted genes suggest that genes might be altered downstream, and it is appropriate to follow-up with genome-wide investigations of gene expression to determine pathways involved.

In this study, genome-wide gene expression was examined in peripheral blood mononuclear cells (PBMC), collected from Holocaust survivor offspring and comparison subjects, to determine whether differentially expressed genes (DEGs) in association with parental Holocaust exposure could be identified. In addition, in view of previous data in this cohort suggesting differential effects for biological markers based on parental exposure, and parental exposure related factors (sex, symptoms, and age at exposure of the exposed parent), these factors were investigated as potential contributors. Finally, the identified signatures were tested to determine the extent to which they mediated the effects of parental Holocaust exposure on functional measures of GC signaling, inflammation, and metabolism.

METHODS

Clinical data collection

Participants. Blood samples from 96 participants (79 Holocaust offspring, 17 controls) who completed blood sampling procedures for the previous study [7, 12] were available for further investigation.

[1]Department of Psychiatry, McLean Hospital, Harvard Medical School, Belmont, MA, USA; [2]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, USA; [3]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [4]Mental Health Care Center, James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA; [5]Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Munich, Germany and [6]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA
Correspondence: Nikolaos P. Daskalakis (ndaskalakis@mclean.harvard.edu) or Rachel Yehuda (rachel.yehuda@va.gov)
These authors contributed equally: Changxin Xu, Heather N. Bader, Chris Chatzinakos

Received: 4 June 2020 Revised: 19 September 2020 Accepted: 22 October 2020
Published online: 10 November 2020

The original study was designed to examine endocrine and molecular correlates of parental PTSD in five groups (Holocaust offspring with and without maternal and/or paternal PTSD, and comparison subjects). The study was approved by the institutional review boards of the James J. Peters Veterans Affairs Medical Center and Icahn School of Medicine at Mount Sinai; all participants provided written, informed consent.

Participants characteristics. Holocaust offspring were conceived after parental Holocaust-related exposure(s), and parents of Jewish controls were living in North America during the Holocaust, as previously described [7, 12]. Axis I diagnoses were determined by clinical psychologists using the Structured Clinical Interview for the DSM-IV as previously described [13]. Participants also completed measures to assess relevant psychiatric symptoms and early life experiences, including the Beck Depression Inventory (BDI) [14], the Spielberger State Trait Anxiety Inventory (STAI) [15], and the Childhood Trauma Questionnaire [16]. Maternal PTSD (M-PTSD) and paternal PTSD (F-PTSD) were determined by consensus of at least three clinicians based on the Parental PTSD Questionnaire [17], initially completed by the offspring, and reviewed in a semi-structured interview regarding parental symptoms.

Primary data generation

Peripheral blood mononuclear cells (PBMCs) isolation and RNA extraction. Morning (8 a.m.) blood samples were collected. Differential white blood cell count were determined by CLIA-certified lab. In addition, PBMCs were purified from EDTA-pretreated blood and RNA, from Trizol-dissolved PBMCs, was extracted using RNeasy Mini Kits (Qiagen) to isolate total RNA. RNA concentration was determined using a ND-1000 spectrophotometer (NanoDrop Technologies). The purity and quality of the extracted total RNA were evaluated using the RNA 6000 Nano LabChip kit and 2100 Bioanalyzer (Agilent Technologies). Samples had high RNA quality with a mean RNA integrity number (RIN) of 9.37 ± 0.56 (SD).

Illumina cDNA microarrays. 250 ng total RNA were used for amplification. Amplification was done with Illumina TotalPrep™-96 RNA Amplification Kit; Ambion Catalog Number 4393543. Microarray Hybridization: Illumina Beadchips Human12v4 have been used as microarrays following the manufacturer's protocol (11322355 Rev. A). Microarrays were scanned with a BeadArray leader (Illumina). Scan configuration had a scan factor of 1 and active outlier detection (on bead level). The resulting BeadSummary data from the scanner are provided as idat files. Conversion into txt files was done in Genome Studio without imputing missing BeadTypes, without normalization and without background subtraction on probe level. The used chips have 12 samples per chip. All amplification reactions and chips were processed in parallel at the same time. All array probes were subjected to quality control (QC). The Illumina Bead Array Reader was used to scan the microarrays and summarized raw probe intensities were exported using Illumina's GenomeStudio v2011.1 Gene Expression module.

Real-time PCR (RT-PCR). RT-PCR was used for determination of *FKBP51* (FKBP5 gene) expression, as previously described [12]. The primers and probes are described in Supplementary Table S1 [excel file]. A TaqMan RT-PCR assay was used for determining *MMP8* expression ordered by Thermo Fisher Scientific (Primer ID: Hs01029061) with GAPDH as the reference gene. Data analysis was performed using qBase v2.5 (Biogazelle NV, Belgium). RT-PCR-based quantifications were compared with microarray-based quantifications.

Functional measures. PBMC functional measures of GC signaling in this cohort have been previously described [7, 10, 12] and included (i) 8 a.m. basal plasma cortisol, (ii) lysozyme suppression test, an in vitro measure of sensitivity to DEX in PBMCs ("$IC_{50\text{-}DEX}$"), (iii) number of methylated cytosine methylation sites at *NR3C1* exon 1F promoter, and (iv) cytosine methylation at the sites of the *FKBP5* intron 7 GC binding sequence. Plasma C-reactive protein (CRP) levels, obtained by a CLIA-certified lab were used as functional measures of inflammation. Plasma functional measures of metabolism included alkaline phosphatase (ALP), total cholesterol, free thyroxine, gamma-glutamyl transferase, glucose, HDL cholesterol, hemoglobin A1c, insulin, LDL cholesterol (calculated), thyroid stimulating hormone, triglycerides (TG), triiodothyronine (T3), and were obtained by a CLIA-certified lab.

Data analyses

cDNA microarray data preanalysis processing. Each transcript was transformed and normalized through variance stabilization and normalization [18]. Technical batches and effects of RIN were adjusted using Supervised Normalization of Microarray [19]. No significant surrogate variable could be identified suggesting that most of the confounding effects were captured by the known batch effects. Probes were annotated using Reannotator [20] according to their probe sequence. 47,323 raw probes were included in the array. 34,674 probes were mapped to 20,413 unique HGNC gene symbols from which we kept the 20,413 probes with the highest average expression per symbol [21]. Genes *RPS4Y1* and *XIST* (i.e., genes on the Y and X chromosomes, respectively) were used for sex determination based on expression data [22]. Two offspring samples failed this QC sex-check step leaving 94 samples for downstream gene expression analyses. Before differential expression analysis, a detection filtering was applied to exclude genes with very low expression: gene symbols without any sample with detection p value < 0.05 were excluded; 17,057 out of 20,413 gene symbols (83.56%) passed that threshold.

Differential gene expression was evaluated using the *limma* package [23] and included:

a. Across all samples (n = 92), the analysis sought to detect relationships between parental Holocaust exposure and gene expression levels. For this analysis we used the following covariates: (i) age and sex, which have wide-spread effects in PBMC gene expression patterns [24, 25]; (ii) mean arterial pressure as an indicator of autonomic state at the time of blood collection [26], which can have wide effects in PBMC functions [27]; (iii) STAI-state and BDI total scores to control for current anxiety and depression symptom severity, respectively, which can influence peripheral gene expression [28]; (iv) the ratio of lymphocytes to monocytes was calculated (PBMC-ratio) as a proxy of PBMC cell type since PBMC composition may affect estimates of gene expression [29]. The use of these covariates necessitated the removal of two subjects leaving $n = 92$ samples for the final analyses (*with the relevant demographic/clinical data in* Supplementary Table S2A [excel file]). We used the default multiple testing correction of p values in *limma*, which is Benjamini Hochberg's False Discovery Rate (FDR). Additional covariates (related to alcohol use, childhood trauma, smoking, and weight) were evaluated to investigate their influence on the results. Since these results revealed almost identical results and the use of all these additional covariates will reduce further our sample size (i.e., these additional covariates were not available for all 92 subjects), we retained the smaller list of covariates.

b. Within Holocaust offspring (n = 77), using the same five covariates of the main analysis, exploratory analyses were performed to investigate the effects of:

(i) maternal Holocaust exposure, controlling additionally for paternal Holocaust exposure (*with the relevant demographic/clinical data in* Supplementary Table S2B [excel file]);
(ii) paternal Holocaust exposure, controlling additionally for maternal Holocaust exposure (*with the relevant demographic/clinical data in* Supplementary Table S2C [excel file]);

(iii) childhood trauma, as accessed by CTQ total score (*with the relevant demographic/clinical data and CTQ total score histogram in* Supplementary Table S2D [excel file]).

c. In offspring with maternal Holocaust exposure (n = 66), using the same five covariates of the main analysis, we additionally explored the effects of:

(i) maternal PTSD, controlling additionally for paternal Holocaust exposure and PTSD (*with the relevant demographic/clinical data in* Supplementary Table S2E [excel file]).
(ii) maternal age at Holocaust exposure (as continuous variable), controlling additionally for paternal Holocaust exposure (*with the relevant demographic/clinical data and the histogram of maternal age at Holocaust exposure in* Supplementary Table S2F [excel file]).

d. In offspring with paternal Holocaust exposure (n = 68), using the same five covariates of the main analysis, we explored the effects of:

(i) paternal PTSD, controlling additionally for maternal Holocaust exposure and PTSD (*with the relevant demographic/clinical data in* Supplementary Table S2G [excel file]).”
(ii) paternal age at Holocaust exposure, controlling additionally for maternal Holocaust exposure (*with the relevant demographic/clinical data and the histogram of paternal age at Holocaust exposure in* Supplementary Table S2H [excel file]).

We also conducted differential gene expression analyses using a nonparametric statistics method, Significance Analysis of Microarrays (SAM) [30]. We performed SAM for all the above primary and secondary outcomes in order to compare the agreement with the results coming from *limma*. SAM results output included SAM score *d*, an FDR-based *q* value, and fold change.

Comparisons of differential gene expression results. To compare results from different genome-wide analyses, we calculated Spearman rank correlations (rho) of the fold-changes (FC). Rank-rank hypergeometric overlap (RRHO) analyses were also performed to identify significant overlap of differential expression lists between pairs of results by determining the degree of statistical enrichment using the hypergeometric distribution [31].

Weighted gene co-expression network analysis (WGCNA). It was of interest to identify gene co-expression networks associated with parental Holocaust exposure because gene expression alterations are often coordinated [32]. WGCNA was used [33] to build unsigned co-expression networks (modules) for the 77 Holocaust offspring samples. We used only the offspring samples so that our modules are not affected by differential gene expression between Holocaust offspring and controls. We used the default parameter settings for unsigned networks (such as, 20 as the minimum module size, Pearson correlation and soft-thresholding power of 6). Once modules were identified, singular value decomposition of each module’s expression matrix was performed for 94 subjects, not only for the Holocaust offspring. The resulting module eigengene (ME), equivalent to the first principal component, was used to represent the overall expression profiles for each module. MEs were analyzed with *limma* as described above to identify differentially expressed modules.

Preservation was also investigated to determine whether the network properties of networks/modules constructed in Holocaust offspring are maintained in comparison subjects’ samples. The WGCNA *modulePreservation* function calculated the module preservation statistics between two datasets: the reference dataset (i.e., Holocaust offspring) and the test dataset (comparison subjects). The key outcome of the above function is the $Z_{summary}$ score per module ($Z_{summary} = \frac{Z_{connectivity} + Z_{density}}{2}$) [34], where $Z_{\text{connectivity}}$ is the median between the three connectivity based statistics $(Z_{cor.kIM}, Z_{cor.kME}, Z_{cor.cor})$ and $Z_{density}$ is the median between the four density preservation statistics $(Z_{meanCor}, Z_{meanAdj}, Z_{propVarExpl}, Z_{meanKME})$. The resulting score is judged according to three possible scenarios: (i) if $Z_{\text{summary}} > 10$ there is strong evidence that the module is preserved, (ii) if $2 < Z_{\text{summary}} < 10$ there is weak/moderate evidence of preservation, and (iii) if $Z_{\text{summary}} < 2$, there is no evidence that the module is preserved.

In order to calculate the genes with the highest connectivity per module we first computed the module adjacency matrix, which is the correlation matrix between every pair of genes belonging to the module, raised to power of 2 (default). Then, the r^2 corresponding to each gene were summed, and all the genes were ordered based on their summation. Genes with high summation were considered top-connected, while the one with the highest is the hub gene [34].

Gene set enrichment analysis (GSEA) [35] implementation in R [36], *fgsea*, was used to test concordance of differential gene expression analyses results with 17,815 gene expression signatures (i.e., gene sets) from: (i) curated gene sets available in Molecular Signatures Database (MSigDB, datasets: http://software.broadinstitute.org/gsea/msigdb); (ii) microarray-based gene expression measured in whole blood before and 3 h after per os 1.5 mg dexamethasone (DEX) administration ($N = 160$) [37]; (iii) RNA sequencing-based expression measured in PBMCs after in vitro culture for 6 h at 0 nM, 2.5, 5, and 50 nM DEX in ten PTSD and ten control subjects [38].

GSEA estimates the enrichment for a gene set of an input gene list, ranked based on differential gene expression, against the enrichments for the same gene set of permuted gene lists of the same size as the input gene list. The default number of permutations is 1000, but to increase robustness of results 10,000 permutations were performed. Furthermore, GSEA is also conducting an FDR adjustment of the permutation-based *p* value to control for the number of the different gene sets tested for enrichment.

For the curation of the extra gene sets from the two DEX-stimulation datasets [37, 38], we took into account the GSEA developers’ recommendation that the permutation test and enrichment score normalization is not very accurate for extremely small or extremely large gene sets; (the recommended maximum for an input gene list of 10,000 to 20,000 genes is 500 genes per gene set). For both datasets we first used a threshold of FDR-adjusted *p* value < 0.05 and then we selected the top 500 ranked genes based on the GSEA ranking metric that is the product of the sign of the ‘direction’ in the expression FC and the *p* value.

Upstream regulator analysis (URA) [39] was performed as described previously [40] on genes with absolute $\log_2$FC >0.1 and *p* value < 0.05. Criteria for detecting the activity of an upstream regulator were: (i) absolute bias-corrected *z*-score more or equal to 1.0; (ii) enrichment FDR-adjusted *p* value < 0.05.

Mediation analyses was conducted for blood functional measures with primary parental Holocaust exposure effects. A model-based mediation was performed using the mediation package in R [41], to quantify the possible mediation effect of (i) Holocaust-associated gene expression on the relationship between parental Holocaust exposure and functional markers; or (ii) Holocaust-associated functional markers on the relationship between parental Holocaust exposure and gene expression. To estimate these mediation effects, we estimated three regression equations. In these linear models, *Y* is the outcome of interest, *X* is the exposure and *M* is the mediator, where *E*(*Y*) and *E*(*M*) are the mean or expected value of *Y* and *M*, respectively [42, 43]: $E(Y) = b_0 + cX + covariates(eq1)$; $E(M) = b'_0 + aX + covariates(eq2)$; and $E(Y) = b'_o\prime + c'X + bM + covariates(eq3)$. *axb* is the indirect (mediation effect), *c*′ is the direct effect and *c* is the total effect (also we can define *c* as $c = axb + c'$). In the event that both estimations of *c* (eq1) and *a* (eq2) were significant, we checked compared the direct effect of *X* on *Y* (controlling for *M*) (*c*′ in eq3) with the total effect of *X* on *Y* (*c* in eq1). A bootstrapping method

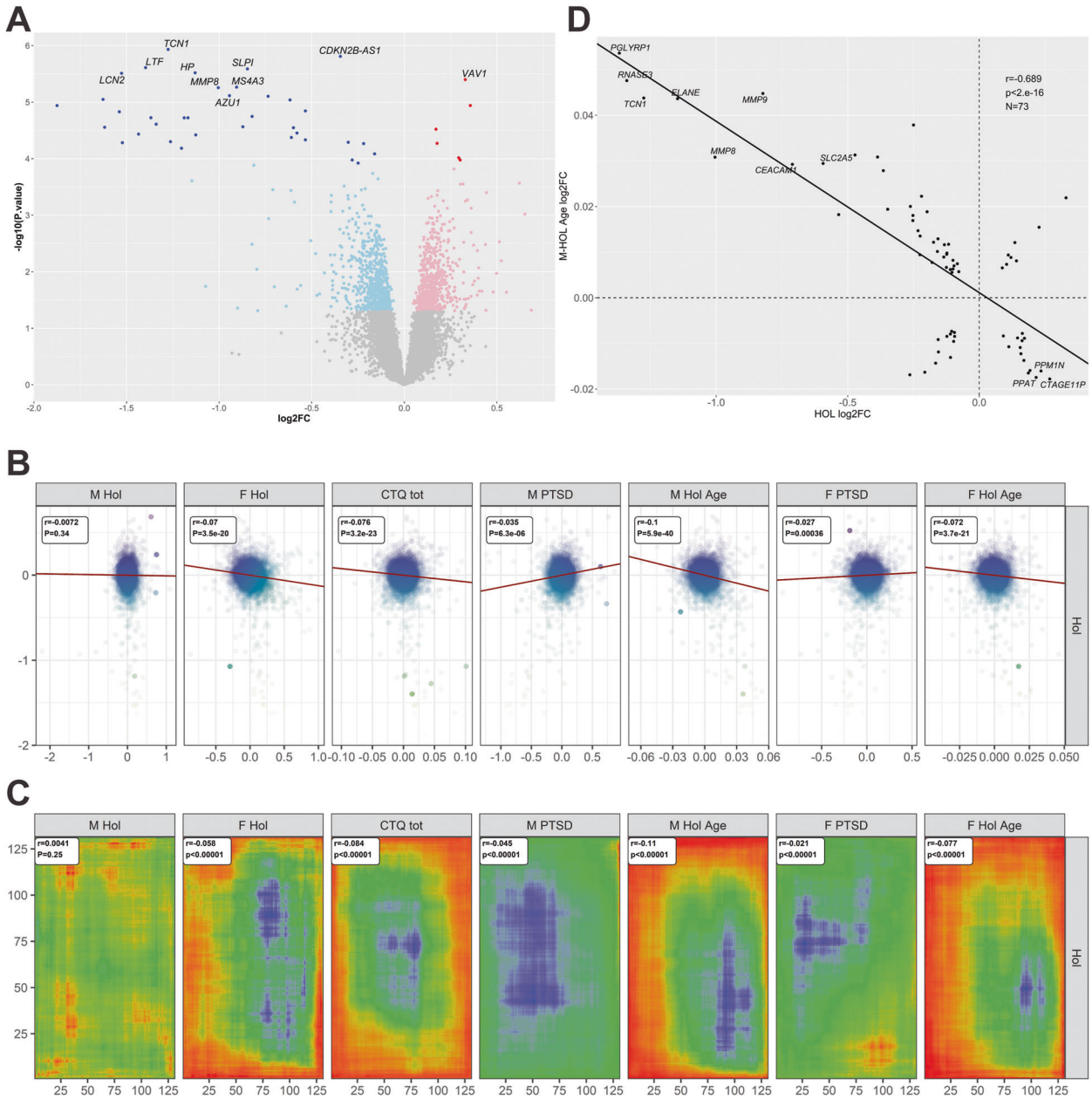

Fig. 1 Differential gene expression associated with parental Holocaust exposure. A Volcano plot illustrating the relationship of *p* value with fold changes (log_2FC) of the differential expression results by parental Holocaust exposure. Blue indicates downregulation, while red indicates upregulation. Bright colors indicate genes with adjusted *p* value < 0.05. The top ten genes with the lowest *p* values have been named. **B** Spearman correlations and **C** rank-rank hypergeometric overlap (RRHO) of differential gene expression fold-changes (log_2FC) based on maternal and paternal Holocaust exposure ("M Hol" and "F Hol", respectively), childhood trauma ("CTQ tot"), maternal and paternal PTSD ("M-PTSD" and "F-PTSD", respectively), and maternal and paternal age at Holocaust exposure ("M Hol Age" and "F Hol Age", respectively) with log_2FC based on parental Holocaust exposure ("Hol"). **D** Spearman correlation of log_2FC from the differential expression analyses based on Hol vs. M Hol Age for genes nominally significant genes in both analyses. Genes with $log_2FC > 1$ SD in both analyses are named.

(10000 iterations) for eq2 and eq3 was used to estimate the significance of the mediation effect. We also used the Sobel test as an alternative method for testing the significance of mediation [44]. Both Sobel method and bootstrapping method had similar results, and we used the minimum p-value between the two tests to prioritize mediation results. A common metric to represent the mediation effect is the mediated proportion (MP), defined as the ratio between a(eq2) x b(eq3) and c (eq1) [45]. Note that, in order for the coefficients to be comparable, we rescaled the *Y*s and *M*s with the min-max normalization method: $x' = \frac{x_i - minX}{\max(X) - \min(X)}$; the *X* was already 0.1.

RESULTS

Differential gene expression signature of parental Holocaust exposure

There were 42 FDR-significant DEGs in association with parental Holocaust exposure, controlling for offspring's age, sex, mean arterial pressure, state anxiety and depression severity, and PBMC-type. Figure 1A depicts the respective Volcano plot (*p* values by fold changes), Table 1A contains the FDR-significant DEGs and Supplementary Table S3A [excel file] contains the full list of DEGs. Most of the FDR-significant DEGs were downregulated (36 out of 42) in the Holocaust offspring group. Adding as additional

Table 1. Differential gene expression associated with Parental Holocaust exposure.

Symbol	Name	Location	Type(s)	logFC	AveExpr	*t*	*P* .Value	adj. *P*. Val	*B*
A DE Genes									
TCN1	Transcobalamin 1	Cytoplasm	Transporter	−1.27558	8.324074	−5.22261	1.17E−06	8.79E−03	4.95309
CDKN2B- AS1	CDKN2B antisense RNA 1	Other	Other	−0.34567	4.770733	−5.15357	1.55E−06	8.79E−03	4.70906
LTF	Lactotransferrin	Extracellular Space	Peptidase	−1.39744	6.999817	−5.04149	2.45E−06	8.79E−03	4.31622
SLPI	Secretory leukocyte peptidase inhibitor	Cytoplasm	Other	−0.84749	4.791383	−5.02864	2.58E−06	8.79E−03	4.27144
HP	Haptoglobin	Extracellular Space	Peptidase	−1.12983	6.438292	−4.98915	3.03E−06	8.79E−03	4.134251
LCN2	Lipocalin 2	Extracellular Space	Transporter	−1.52705	8.957932	−4.98387	3.09E−06	8.79E−03	4.11593
VAV1	Vav guanine nucleotide exchange factor 1	Nucleus	Transcription regulator	0.328953	8.757575	4.919992	3.99E−06	9.73E−03	3.89527
MS4A3	Membrane spanning 4-domains A3	Plasma Membrane	Other	−0.90588	9.725272	−4.84303	5.43E−06	1.05E−02	3.631
MMP8	Matrix metallopeptidase 8	Extracellular Space	Peptidase	−1.0047	4.548622	−4.83734	5.55E−06	1.05E−02	3.61195
AZU1	Azurocidin 1	Cytoplasm	Peptidase	−0.94434	6.306243	−4.75552	7.68E−06	1.20E−02	3.333998
ABCA13	ATP binding cassette subfamily A member 13	Extracellular Space	Transporter	−0.73573	4.415375	−4.74899	7.88E−06	1.20E−02	3.311951
CEACAM8	Carcinoembryonic antigen related cell adhesion molecule 8	Plasma Membrane	Other	−1.62709	7.817593	−4.7165	8.95E−06	1.20E−02	3.202367
PRTN3	Proteinase 3	Extracellular Space	Peptidase	−0.61767	4.930315	−4.71086	9.15E−06	1.20E−02	3.18339
COL24A1	Collagen type XXIV alpha 1 chain	Extracellular Space	Other	0.356141	5.596303	4.65272	1.15E−05	1.31E−02	2.98848
DEFA1B	Defensin alpha 1	Cytoplasm	Other	−1.87564	9.765095	−4.65206	1.15E−05	1.31E−02	2.98629
PCOLCE2	Procollagen C-endopeptidase enhancer 2	Extracellular Space	Other	−0.53453	4.762042	−4.59598	1.43E−05	1.48E−02	2.79958
CEACAM6	Carcinoembryonic antigen related cell adhesion molecule 6	Plasma Membrane	Other	−1.53924	6.948948	−4.58721	1.48E−05	1.48E−02	2.770507
COL17A1	Collagen type XVII alpha 1 chain	Extracellular Space	Other	−0.82222	5.510827	−4.53745	1.79E−05	1.54E−02	2.606086
PGLYRP1	Peptidoglycan recognition protein 1	Plasma Membrane	Transmembrane receptor	−1.36851	6.810638	−4.52405	1.89E−05	1.54E−02	2.56200
BPI	Bactericidal/permeability-increasing protein	Plasma Membrane	Transporter	−1.16851	6.97071	−4.52313	1.89E−05	1.54E−02	2.558957
ARG1	Arginase 1	Cytoplasm	Enzyme	−1.18879	5.943598	−4.52274	1.90E−05	1.54E−02	2.557698
RNASE3	Ribonuclease A family member 3	Extracellular Space	Enzyme	−1.34025	7.244765	−4.45522	2.45E−05	1.90E−02	2.33669
TFF3	Trefoil factor 3	Extracellular Space	Other	−0.87215	5.201223	−4.42749	2.73E−05	1.94E−02	2.246498
TACSTD2	Tumor associated calcium signal transducer 2	Plasma Membrane	Other	−1.61862	5.92066	−4.42213	2.78E−05	1.94E−02	2.229096
GOLGA8S	Golgin A8 family member S	Other	Other	−0.59892	4.888	−4.41691	2.84E−05	1.94E−02	2.212192
PAGE3	PAGE family member 3	Other	Other	0.170903	4.720445	4.40091	3.01E−05	1.98E−02	2.16037
CEBPE	CCAAT/enhancer binding protein epsilon	Nucleus	Transcription regulator	−0.57991	5.269775	−4.35971	3.52E−05	2.22E−02	2.027484
CAMP	Cathelicidin antimicrobial peptide	Cytoplasm	Other	−1.43526	9.773283	−4.3482	3.67E−05	2.23E−02	1.990475
OLR1	Oxidized low density lipoprotein receptor 1	Plasma Membrane	Transmembrane receptor	−1.12642	5.875011	−4.33967	3.79E−05	2.23E−02	1.96311
SERPINB10	Serpin family B member 10	Cytoplasm	Other	−0.61022	5.338705	−4.31163	4.21E−05	2.40E−02	1.87333
CRISP3	Cysteine rich secretory protein 3	Extracellular Space	Other	−0.5345	4.553876	−4.28574	4.64E−05	2.55E−02	1.790759
CTSG	Cathepsin G	Cytoplasm	Peptidase	−1.26287	7.733624	−4.26548	5.00E−05	2.56E−02	1.72638
TRHDE–AS1	TRHDE antisense RNA 1	Other	Other	−0.30311	4.707297	−4.25861	5.13E−05	2.56E−02	1.70459
DEFA4	Defensin alpha 4	Extracellular Space	Other	−1.52279	8.574051	−4.25429	5.21E−05	2.56E−02	1.690901
MIR339	Microrna 339	Cytoplasm	microRNA	0.176806	4.852657	4.246782	5.36E−05	2.56E−02	1.66711
MPST	Mercaptopyruvate sulfurtransferase	Cytoplasm	Enzyme	−0.22	7.124978	−4.24442	5.41E−05	2.56E−02	1.65963
ANXA3	Annexin A3	Cytoplasm	Enzyme	−1.2035	6.076999	−4.19279	6.54E−05	3.02E−02	1.496881
HSD17B2	Hydroxysteroid 17-beta dehydrogenase 2	Cytoplasm	Enzyme	−0.16079	4.718598	−4.13098	8.20E−05	3.68E−02	1.30372

Table 1. continued

Symbol	Name	Location	Type(s)	logFC	AveExpr	*t*	*P* .Value	adj. *P*. Val	*B*
FZD3	Frizzled class receptor 3	Plasma Membrane	G-protein coupled receptor	0.293411	5.654598	4.086534	9.64E–05	4.21E–02	1.165946
ZNF8	Zinc finger protein 8	Nucleus	Other	–0.28163	5.046322	–4.06115	1.06E–04	4.39E–02	1.0877
ELP6	Elongator acetyltransferase complex subunit 6	Other	Other	0.301306	8.333097	4.061151	1.06E–04	4.39E–02	1.087698
GRIP2	Glutamate receptor interacting protein 2	Plasma Membrane	Other	–0.24957	4.695892	–4.02654	1.20E–04	4.86E–02	0.981522
B DE Co–expression Modules									
N/A	ME chocolate	N/A	WGCNA module	↓	N/A	–4.71168	7.41E–06	0.000385	3.416518

Gene members (FDR-significant DEGs underlined): ABCA13,ANXA3,ARG1, ARHGAP5,AZU1,BPI,CAMP, CD24, CEACAM1,CEACAM6, CEACAM8,CEBPE, CHI3L1, CHIT1,COL17A1, CRIM1,CRISP3, CTCF,CTSG, DEFA1B, DEFA4, ELANE,ELP6, FOXP3,GOLGA8S, GRIP2, H1FNT, HMGB3,HP, IGBP1P1, KIF2C,LCN2, LTF, MDK,MMP8, MMP9, MPO, MYB, MYMK, OLFM4,OLR1, OR4K15, ORM1, ORM2, OSTCP1,PCOLCE2, PGLYRP1, PRTN3, PTPRD, RETN,RNASE3, RNF4, S100P,SERPINB10, SLC28A3, SLC2A5,SLPI, SPRR2D,TACSTD2, TAF2, TESMIN,TFF3, TRHDE-AS1, USP18,VAV1 Hub gene:LCN2

covariates alcohol use, BMI, childhood trauma (i.e., CTQ total score) or current smoking resulted in very similar effect sizes (rho of FCs > 0.94).

We used the same RNA used for the microarrays for technical validation of *FKBP5* gene expression by RT-PCR. RT-PCR based *FKBP5* expression was positive correlated with the respective microarray-based signal ($r = 0.55$; Supplementary Fig. S1a). To assess the strength of this positive correlation, RT-PCR based *FKBP5* values were correlated with the microarray-based signals of all the other 17506 genes and revealed as expected that the *FKBP5-to-FKBP5* correlation was the highest, corresponding to a permutation-based p value = 5.86e−05 (Supplementary Fig. S1b).

Additional differential gene expression signatures

Exploratory analyses were conducted *within Holocaust offspring* to detect additional effects of Holocaust exposure (Supplementary Table S3B [excel file] and Supplementary Table S3C [excel file], respectively), childhood trauma (Supplementary Table S3D [excel file]), maternal PTSD and age at Holocaust exposure (Supplementary Table S3E [excel file] and Supplementary Table S3F [excel file], respectively), and paternal PTSD and age at Holocaust exposure (Supplementary Table S3G [excel file] and Supplementary Table S3H [excel file], respectively). The seven Volcano plots can be found in Supplementary Fig. S2 in the same order. Significant interrelationships were observed (Supplementary Fig. S3). Spearman rank correlation (Fig. 1B) and RRHO overlap analyses (Fig. 1C) revealed a small degree of relationship with the main analysis, based on Holocaust exposure; the highest correlation was observed for maternal age at Holocaust exposure (rho = −0.10—rrho $r = -0.11$). This correlation was driven by 11 genes (out of the 73 nominally significant in both analyses) with the larger effect sizes, which were named in Fig. 1D and included four FDR-significant DEGs (downregulated by parental Holocaust exposure).

For both maternal and paternal Holocaust exposure analyses, the strongest correlation and overlap were between each other (rho = 0.21—rrho $r = 0.23$), driven by 18 genes (out of 88 nominally significant in both analyses—Fig. 2A). For both maternal and paternal PTSD analyses the strongest correlation and overlap were between each other and it was in the opposite direction (rho = −0.29—rrho $r = -0.30$), driven by ten genes (out of 66 nominally significant in both analyses—Fig. 2B). For both maternal age and paternal age at Holocaust exposure age the strongest correlation and overlap were between each other (rho = 0.66—rrho $r = 0.67$), driven by 41 genes (out of 202 nominally significant in both analyses—Fig. 2C). Finally, for CTQ total score analysis showed the strongest correlation and overlap with paternal PTSD (rho = 0.21—rrho $r = 0.21$), driven by nine genes (out of 23 nominally significant in both analyses—Fig. 2D).

SAM analysis

The nonparametric statistics-based analysis produced almost identical results for the main (rho of FCs and statistics = 0.99. Supplementary Fig. S4a, b, respectively) and additional analyses (rhos > 0.95). For parental Holocaust exposure, FDR-significant genes in both analyses were 35, while seven genes were significant only in *limma* analysis and seven genes only in SAM (Supplementary Fig. S4c).

Gene co-expression network analysis

WGCNA detected 8019 co-expressed genes that were part of 52 gene co-expression modules (Supplementary Fig. S5a, b, Supplementary Table S4A [excel file]). Preservation analysis revealed that most modules were well preserved between the two groups (Supplementary Fig. S5c, d), confirming our strategy to construct the co-expression network using only the Holocaust offspring samples. Only one module (named by WGCNA as Chocolate) had eigengene that significantly differed between Holocaust survivor offspring and comparison subjects (Table 1B, Supplementary

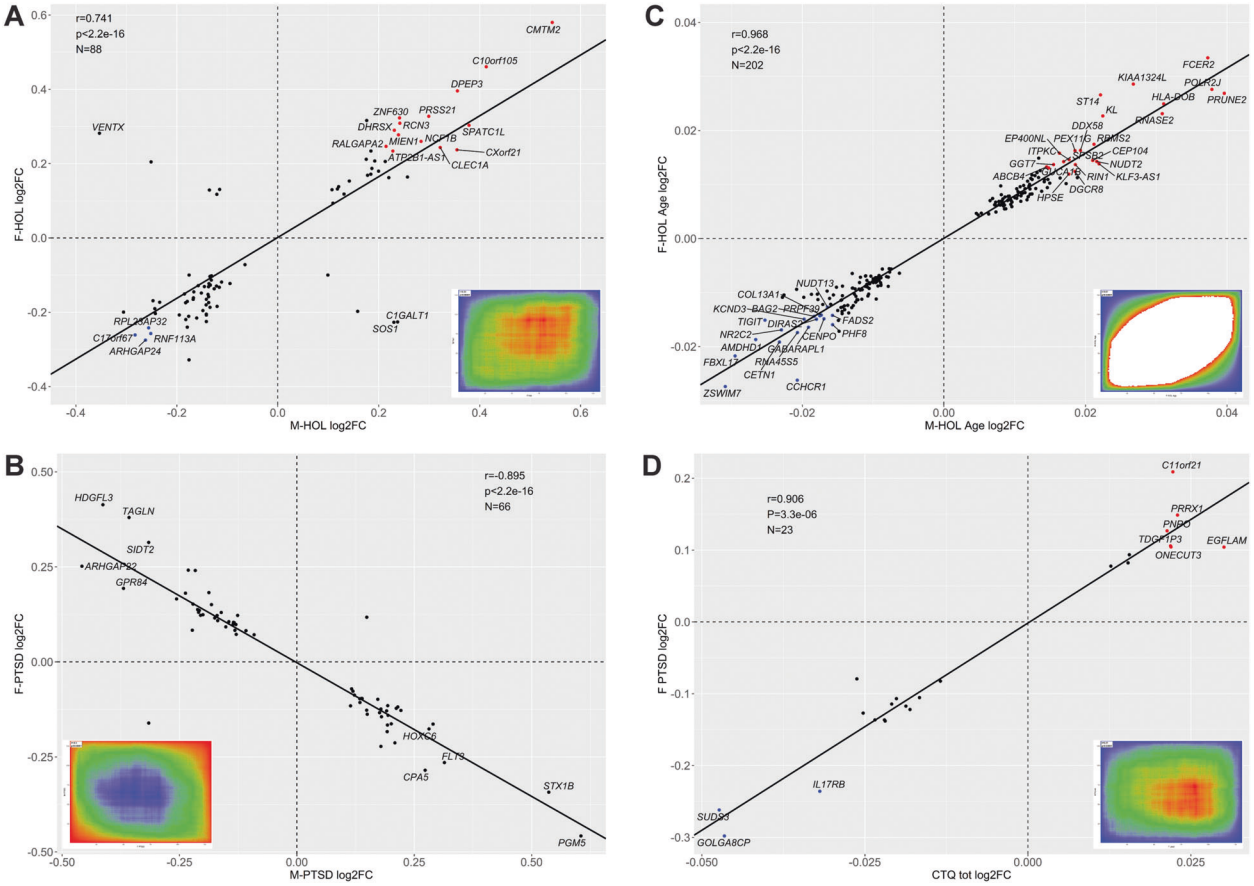

Fig. 2 Significant relationships between additional differential gene expression analyses. Spearman correlation and rank-rank hypergeometric overlap (as an insert) of fold-changes (log_2FC) from the differential expression analyses based on **A** maternal and paternal Holocaust exposure ("M Hol" and "F Hol", respectively), **B** maternal and paternal PTSD ("M-PTSD" and "F-PTSD", respectively) **C** maternal and paternal age at Holocaust exposure ("M Hol Age" and "F Hol Age", respectively), and **D** F-PTSD and childhood trauma ("CTQ tot"). Only genes nominally significant in both analyses of each pair are depicted, and genes with log_2FC >1 SD in both analyses are named.

Table S5B [excel file]), while this module was preserved in both groups ($Z_{summary} = 30.34$). Importantly, this Holocaust-associated module contained 65 genes, including 32 out of the 42 DEGs described above (76.2%), and was enriched for immune response gene ontologies (GO) (Supplementary Table S4C [excel file]; Bonferroni-adjusted $p < e{-}4$). The hub gene of Chocolate module was *LCN2* (Table 1, Supplementary Table S4D [excel file]; Supplementary Fig. S6).

Enrichment analyses

GSEA of DEGs associated with parental Holocaust exposure for 17,815 gene sets (Supplementary Table S5A [excel file]) revealed the strongest enrichment signal for a gene set (Fig. 3A) derived from peripheral blood containing the genes upregulated 3 h after a *per os* administration of DEX [37]. The GC signature was negatively enriched in Holocaust vs. control samples, as indicated by the negative normalized enrichment score (NES, Fig. 3B). In addition, when focusing on the genes affected by both parental Holocaust exposure and maternal age at Holocaust exposure in the opposite direction (Supplementary Table S5B [excel file], Fig. 3C), the top gene set was a negative enrichment of the GO immune response gene set in Holocaust *offspring (vs. controls)* and offspring with *younger (vs. older)* maternal age at Holocaust exposure. Gene sets enriched in other correlated gene signatures can be found in Supplementary Table S5C–F [excel file]).

Moreover, URA of the parental Holocaust DEG signature predicted molecules and chemicals as upstream regulators (Supplementary Table S6 [excel file]). The top-10 predicted regulators: Dexamethasone (DEX, activity direction: ↓, enrichment FDR-adjusted *p* value: 2.21e-12), Myocardin-related transcription factor A and B (MRTFA and MRTFB, ↑, 8.06E-10 and 1.93e-09, respectively), lipopolysaccharide (LPS, ↓, 4.98e-09), GATA-binding factor 2 (GATA2, ↓, 2.48e-08), Colony Stimulating Factor 3 (CSF3, ↓, 1.08e-07), CCAAT enhancer binding protein alpha and epsilon (CEBPA and CEBPE, ↓, 1.06e-07 and 1.54e-07, respectively), Serum Response Factor (SRF, ↑, 3.24e-07), and Interleukin 6 (IL6, ↓ 1.69e-06). The URA enrichment for DEX-regulated transcripts came from an unbiased approach and was driven by 45 genes, which included 13 FDR-significant DEGs (all downregulated). The direction of this effect was in line with low GC tone.

Relationship of parental Holocaust exposure gene signature with basal cortisol

Our differential gene expression analysis picked up genes that are characteristic of the time-point (8 a.m.) we are sampling (i.e., temporally near the circadian peak of cortisol [46]). To explore this observation, we used 8 a.m. plasma cortisol obtained for the same subjects, to generate Spearman correlations of cortisol with the expression levels for: (i) all genes ($n = 17057$); (ii) the genes belonging to "Arloth DST DOWN" gene set [37] (i.e., down-regulated genes by dexamethasone stimulation); and (iii) the expression levels of the genes belonging to "Arloth DST UP" gene set [37] (i.e., downregulated genes by dexamethasone stimulation). Compared to the correlation of all the genes, there was a left shift for the Arloth DST DOWN genes, and a right shift for the Arloth DST UP genes as expected (Supplementary Fig. S7a).

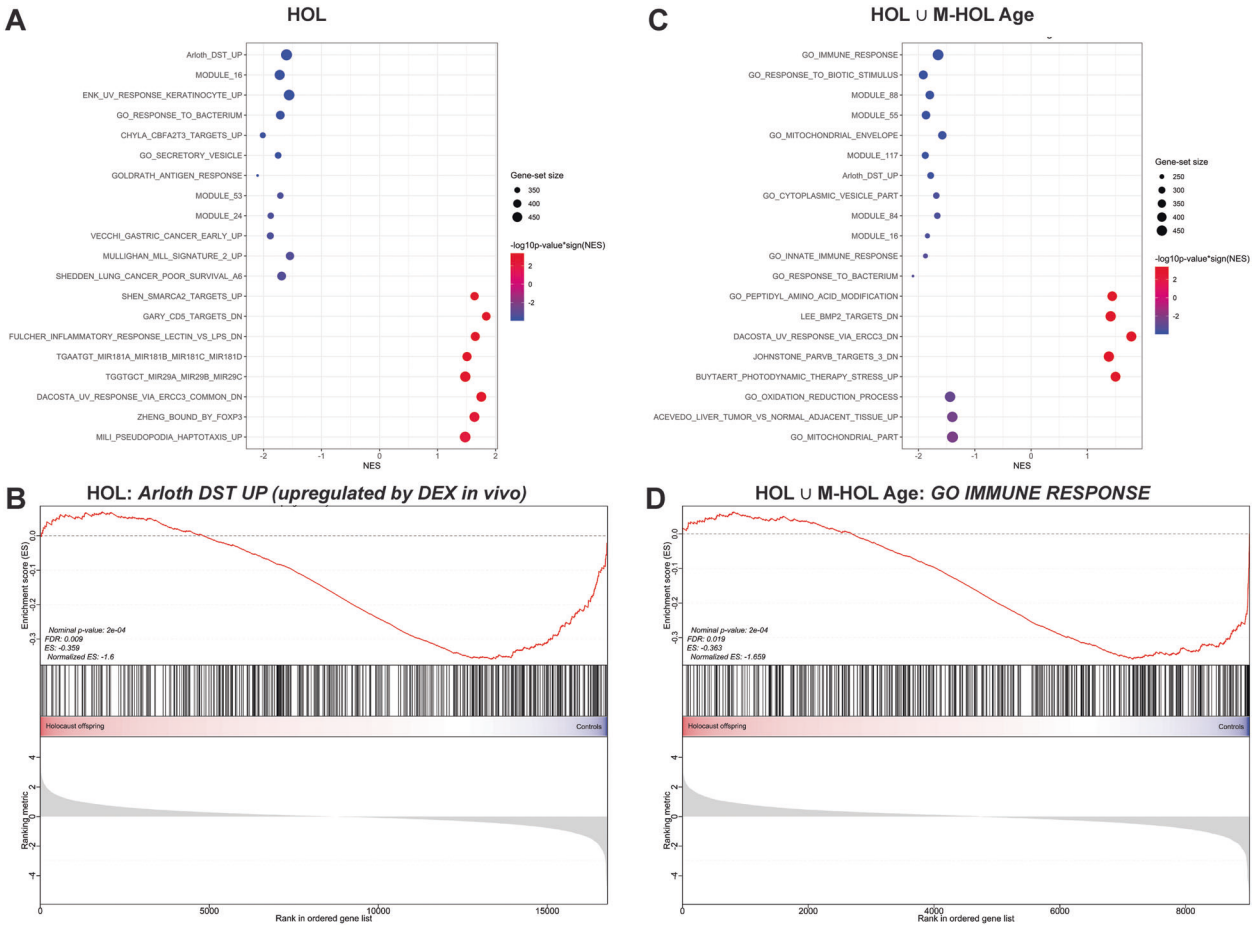

Fig. 3 Gene set enrichment analysis. Buble plots depicting the Top-20 enriched gene-sets of expression signatures based on parental Holocaust exposure (**A**) and genes differentially expressed by both parental Holocaust exposure and maternal age at Holocaust exposure (**C**). GSEA plots and heatmaps of expression signatures by parental Holocaust exposure overlapping with gene set derived based on in vivo DEX stimulation (**B**) and GO Immune Response (**D**). The *y*-axis represents enrichment score (ES) and the *x*-axis (bar-code plot) indicates the position of the genes on the expression data rank-sorted (boxplot) by its association with parental Holocaust exposure (i.e., Holocaust offspring vs. controls), with red and blue colors indicating up- and downregulation of mRNA. ES is the maximum deviation from zero as calculated for each gene going down the ranked list and represents the degree of over-representation of a gene set at the top or the bottom of the ranked gene list.

In addition, the two groups of genes had a significant difference (Supplementary Fig. S7b). However, these relationships did not likely affect the differential gene expression based on parental Holocaust exposure: (i) the two groups did not differ in 8 a.m. plasma cortisol; (ii) when we added 8 a.m. cortisol as an additional covariate in our differential gene expression analysis for parental Holocaust exposure, the result was highly correlated with the analysis without the cortisol covariate (rho of FCs $r = 0.999$).

Mediation analysis

Significant effects of parental Holocaust exposure were detected for five of the functional markers (Supplementary Table S7A [excel file]): number of methylated cytosine methylation sites at *NR3C1* exon 1F promoter ($p = 6.94e\text{-}03$), ALP ($p = 1.41e\text{-}02$), maximum cytosine methylation at the sites of the *FKBP5* intron 7 GC binding sequence ($p = 2.70e\text{-}02$), CRP ($p = 4.03e\text{-}02$), and T3 ($p = 4.71E\text{-}02$).

Mediation analysis revealed that 17 of the FDR-significant DEGs, associated with parental Holocaust exposure, mediated a portion (range 45–78%) of the parental Holocaust exposure effect on CRP controlling for number of genes tested per mediation per functional measure (Supplementary Table S7B [excel file]). One of them met overall FDR-significance: *GRIP2* (Fig. 4A). All genes, but *one*, were members of the Holocaust-associated co-expression network (i.e., Chocolate module). In concordance with the mediation effects by individual genes, the Chocolate module mediated 58.84% of the parental Holocaust exposure effects on CRP (Fig. 4B).

No FDR significant effects were observed in analyses examining the opposite direction of mediation (i.e., functional measures mediating parental Holocaust exposure effects on gene expression, Supplementary Table S7C [excel file]).

Convergent gene alterations in MMP8. In Venn diagram in Fig. 4C we depict the overlap of (i) FDR-significant DEGs based on *limma and SAM*, (ii) genes that were members of the Chocolate module, (iii) DEGs that were in the opposite direction in the analysis based maternal age at Holocaust exposure; (iv) DEGs that regulated by DEX; and (v) mediating effects of parental Holocaust exposure on blood functional measures. The full lists of genes and their intersections are in Supplementary Table S8 [excel file]). There was only one gene, *MMP8*, encoding for matrix metalloproteinase-8, was found in all these categories, while another metalloproteinase, *MMP9*, was found in 3 categories. Note also that *MMP8* was one of the top connected genes in the Chocolate module (above the 75th percentile, Supplementary Fig. S6).

MMP8 validation. For 50 subjects, using frozen PBMCs that were not used for the microarray experiments, RNA was extracted.

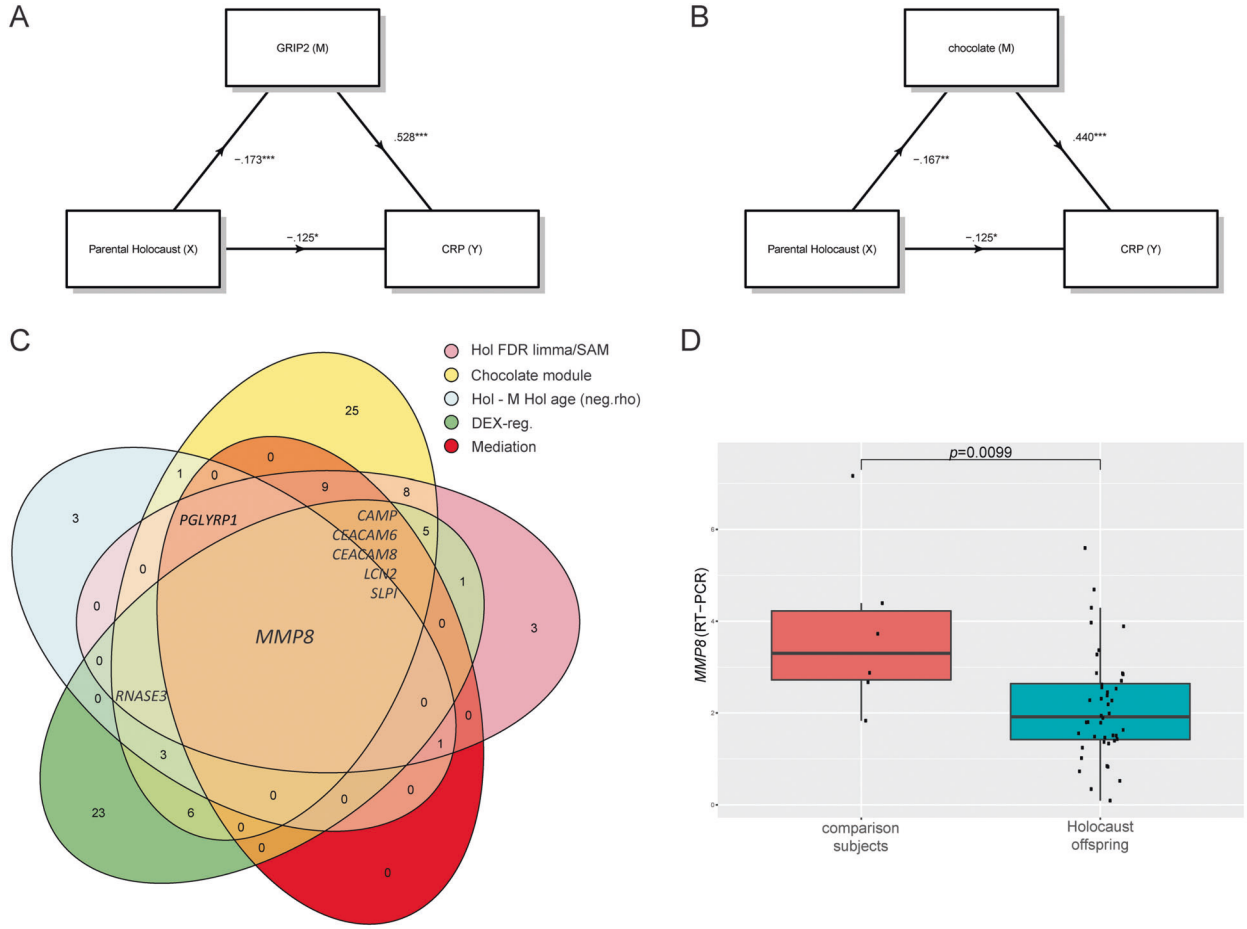

Fig. 4 Mediation analysis and tope-genes. A Mediation: *GRIP2* gene mediated 73.1% of the effect of parental Holocaust exposure on CRP (p=2e−04). **B** Mediation: Chocolate co-expression network mediated 58.8% of the effect of parental Holocaust exposure on CRP ($p = 0.0042$). **C** Venn diagram depicts the overlap of (i) FDR-significant DEGs, (ii) genes that were members of the Chocolate module, (iii) DEGs that were in the opposite direction in the analysis based maternal age at Holocaust exposure; (iv) DEGs that regulated by DEX; and (v) mediating effects of parental Holocaust exposure on blood functional measures. **D** Validation: box plot of RT-PCR based *MMP8* expression in 50 subjects: comparison subjects ($n = 6$ in red) compared to Holocaust offspring ($n = 44$ in green). *P* value of the group difference was estimated by a Wilcoxon signed rank test.

The RT-PCR based gene expression of the FDR-significant *MMP8* showed positive correlation with the respective microarray-based values ($r = 0.59$; Supplementary Fig. S8a). To assess the strength of the correlation, RT-PCR based *MMP8* values were correlated with the microarray-based signals of all the other 17506 genes and revealed as expected that the *MMP8-to-MMP8* correlation was the 4th highest, corresponding to a permutation-based *p* value 2.9e-04 (Supplementary Fig. S8b). Importantly, the MMP8 association with parental Holocaust exposure was significant and in the same direction (Fig. 4D).

DISCUSSION

This is the first study demonstrating that parental Holocaust exposure can be associated with blood genome-wide gene expression patterns in adult offspring and included 42 genes with FDR-adjusted significance. Most of these genes were downregulated and co-expressed, and pathway enrichment analyses revealed that were related to low GC signaling and innate immunity. Both these pathways have been have been described in relation to the intergenerational transmission of stress effects [6, 7, 47, 48]. Previously, blood differential genome-wide DNAm was observed in offspring exposed prenatally to the Dutch famine [49], while limited portion of DNAm loci associated with childhood malnutrition showed evidence for intergenerational transmission in another study [50].

Additional gene expression alterations were associated with factors related to parental Holocaust exposure such as sex of exposed parent, parental PTSD, and parental age at exposure. Analysis based on maternal age at exposure produced the most concordant gene expression results with parental Holocaust exposure, reminiscent with the concordance of results of two blood-based GC factors: for DNAm at a intron 7 distal enhancer region of *FKBP5* gene in blood DNA [10, 11] and 11β-Hydroxysteroid dehydrogenase (11β-HSD-2) activity in plasma [8]. Genes that were affected by both M-PTSD and F-PTSD were regulated in the opposite direction. Previously, we have reported that DNAm at the GR exon 1F promoter was differentially affected by M-PTSD (↓) and F-PTSD (↑) [12]. The strong relationship of expression changes based on maternal and paternal age at Holocaust exposure is not a surprise based on their high positive correlation as variables ($r > 0.80$). Finally, concordance in the biological alterations in relation to childhood trauma and F-PTSD was reported before for DNAm at the GR exon 1F promoter [12, 51].

The top gene in this study was *MMP-8* which was FDR-significantly downregulated in Holocaust offspring (compared to controls) and in Holocaust offspring whose mothers were young

when exposed to Holocaust. In addition, this gene is a member of the Holocaust-associated co-expression module, regulated by glucocorticoids and mediated the effects of Holocaust on CRP. MMP-8 is a collagenase and together with the other metalloproteinases are involved in extracellular matrix degradation, as well as stress-regulated wound healing and inflammation [52–54]. Interestingly, another metalloproteinase, MMP-9 (downregulated), and two collagen genes (*COL17A1*: downregulated, *COL24A1*: upregulated) were found in our FDR-significant genes. Extracellular matrix and collagen gene-sets were negatively enriched in PVN in a model of intergenerational transmission of parental stress effects [55].

The identified gene signatures mediated the effects of parental Holocaust exposure on an inflammatory marker (i.e., CRP). The strongest mediation effect was found for *GRIP2* gene, which encodes for an anchoring protein of AMPA receptor. In a recent genome-wide investigation, DNAm loci mediated a considerable proportion of the associations between of prenatal famine exposure with later-life adiposity and serum TG levels [56]. Another gene worth noticing is the gene encoding 17β-HSD2 enzyme, which is involved in inactivation of androgens and estrogens. A proposed mechanism for intergenerational effects of trauma involves de-masculinization through epigenetic marks introduced during the initial period of in utero development, when the placenta is highly susceptible to maternal stress [57, 58].

A limitation of the study is the small size of the control group. The validity of the main analysis is supported by the biological plausibility of the results, replication of findings using non-parametric statistics, and the concordance with DNAm findings in the same cohort [4, 11, 12, 51]. Moreover, the discovery of GC responsive pathways as top-enriched calls for future investigations to serially sample blood for expression assays before and after cortisol peaks (example in [59]) to differentiate between differences in glucocorticoid tone and transcriptional responsiveness to glucocorticoid fluctuations. An additional limitation is the use microarray-based method instead of sequencing-based method to access genome-wide gene expression. In this study we were very rigorous on detection thresholds and probe reannotation to remove any ambiguous microarray signals following up on our prior work [20, 37]. RT-PCR of two genes technically validated the expression values obtained by microarrays (i.e., significant positive correlation). Importantly, MMP-8 was differentially expressed in both assays. Finally, our analyses were not causative and, thus, we refrained from discussing putative mechanisms.

To conclude, this study identified a set of genes in association with parental Holocaust exposure that are linked with low GC signaling and innate immunity in concordance with two decades of work supporting GC alterations in Holocaust offspring [1]. Most importantly, the identified genes, which included metalloproteinases, were linked with functional inflammatory signals.

FUNDING AND DISCLOSURE

This work was supported by RC1MH088101 "Identification of an Epigenetic Risk Marker for PTSD" from the National Institute of Mental Health and in part by a grant (M01RR00071) for the Icahn School of Medicine at Mount Sinai General Clinical Research Center from the National Institute of Health. The NIMH and NIH had no further role in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

NPD has held a part-time paid position at Cohen Veterans Bioscience. NPD has been a consultant for Sunovion Pharmaceuticals and is on the scientific advisory board for Sentio Solutions. EBB is co-inventor of the European patent application FKBP5: a novel target for antidepressant therapy European Patent# EP 1687443 B1 and receives a research grant from Böhringer Ingelheim to investigate FKBP5 as a candidate target in psychiatric disorders. RY is a co-inventor of the following patent: Genes associated with posttraumatic-stress disorder. WO 2010029176 A1". The remaining authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

NPD, EBB, and RY designed the study. HB coordinated the clinical aspects of the study. AL, LMB, and RY supervised clinical aspects of the study. NPD, PW, and IM performed the wet lab experiments. NPD, EBB and RY supervised the wet lab experiments. NPD designed data analyses. NPD and CX performed primary data analyses. NPD, CX, and CC preformed secondary data analyses. NPD wrote the original draft of the manuscript. All authors reviewed and edited all the manuscript versions.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-020-00900-8).

REFERENCES

1. Yehuda R, Lehrner A. Intergenerational transmission of trauma effects: putative role of epigenetic mechanisms. World Psychiatry. 2018;17:243–57.
2. Yahyavi ST, Zarghami M, Marwah U. A review on the evidence of transgenerational transmission of posttraumatic stress disorder vulnerability. Braz J Psychiatry. 2014;36:89–94.
3. Field NP, Muong S, Sochanvimean V. Parental styles in the intergenerational transmission of trauma stemming from the Khmer Rouge regime in Cambodia. Am J Orthopsychiatry. 2013;83:483–94.
4. Roth M, Neuner F, Elbert T. Transgenerational consequences of PTSD: risk factors for the mental health of children whose mothers have been exposed to the Rwandan genocide. Int J Ment Health Syst. 2014;8:12.
5. Shmotkin D, Shrira A, Goldberg S, Palgi Y. Resilience and vulnerability among aging holocaust survivors and their families: an intergenerational overview. J Intergeneration Relationsh. 2011;9:7–21.
6. Yehuda R, Bierer LM, Schmeidler J, Aferiat DH, Breslau I, Dolan S. Low cortisol and risk for PTSD in adult offspring of holocaust survivors. Am J Psychiatry. 2000;157:1252–9.
7. Lehrner A, Bierer LM, Passarelli V, Pratchett LC, Flory JD, Bader HN, et al. Maternal PTSD associates with greater glucocorticoid sensitivity in offspring of Holocaust survivors. Psychoneuroendocrinology. 2014;40:213–20.
8. Bierer LM, Bader HN, Daskalakis NP, Lehrner AL, Makotkine I, Seckl JR, et al. Elevation of 11beta-hydroxysteroid dehydrogenase type 2 activity in Holocaust survivor offspring: evidence for an intergenerational effect of maternal trauma exposure. Psychoneuroendocrinology. 2014;48:1–10.
9. Daskalakis NP, Lehrner A, Yehuda R. Endocrine aspects of post-traumatic stress disorder and implications for diagnosis and treatment. Endocrinol Metab Clin North Am. 2013;42:503–13.
10. Yehuda R, Daskalakis NP, Bierer LM, Bader HN, Klengel T, Holsboer F, et al. Holocaust exposure induced intergenerational effects on FKBP5 methylation. Biol Psychiatry. 2016;80:372–80.
11. Bierer LM, Bader HN, Daskalakis NP, Lehrner A, Provencal N, Wiechmann T, et al. Intergenerational effects of maternal holocaust exposure on FKBP5 methylation. Am J Psychiatry. 2020;177:744–53.
12. Yehuda R, Daskalakis NP, Lehrner A, Desarnaud F, Bader HN, Makotkine I, et al. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. Am J Psychiatry. 2014;171:872–80.
13. Spitzer RL, Williams JBW, Gibbon M. Structured clinical interview for DSM-IV (SCID). New York State Psychiatric Institute Biometrics Research. 1995.
14. Beck AT. A systematic investigation of depression. Compr Psychiatry. 1961;2:163–70.
15. Spielberger CD. The state-trait anxiety inventory (S T A I): test manual for Form X. Consulting Psychologists Press: Palo Alto; 1968.
16. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl. 2003;27:169–90.

17. Yehuda R, Labinsky E, Tischler L, Brand SR, Lavin Y, Blair W, et al. Are adult offspring reliable informants about parental PTSD? A validation study. Ann NY Acad Sci. 2006;1071:484–7.
18. Lin SM, Du P, Huber W, Kibbe WA. Model-based variance-stabilizing transformation for Illumina microarray data. Nucleic Acids Res. 2008;36:e11.
19. Mecham BH, Nelson PS, Storey JD. Supervised normalization of microarrays. Bioinformatics. 2010;26:1308–15.
20. Arloth J, Bader DM, Roh S, Altmann A. Re-annotator: annotation pipeline for microarray probe sequences. PLoS One. 2015;10:e0139516.
21. Miller JA, Cai C, Langfelder P, Geschwind DH, Kurian SM, Salomon DR, et al. Strategies for aggregating gene expression data: the collapseRows R function. BMC Bioinformatics. 2011;12:322.
22. Staedtler F, Hartmann N, Letzkus M, Bongiovanni S, Scherer A, Marc P, et al. Robust and tissue-independent gender-specific transcript biomarkers. Biomarkers. 2013;18:436–45.
23. Smyth GK. Limma: linear models for microarray data. Bioinformatics and computational biology solutions using R and Bioconductor. Springer; 2005. p. 397–420.
24. Steegenga WT, Boekschoten MV, Lute C, Hooiveld GJ, de Groot PJ, Morris TJ, et al. Genome-wide age-related changes in DNA methylation and gene expression in human PBMCs. Age. 2014;36:9648.
25. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, et al. Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci USA. 2003;100:1896–901.
26. Wehrwein EA, Joyner MJ. Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. Handb Clin Neurol. 2013;117:89–102.
27. Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system (1987-2007). Brain Behav Immun. 2007;21:736–45.
28. Ciobanu LG, Sachdev PS, Trollor JN, Reppermund S, Thalamuthu A, Mather KA, et al. Differential gene expression in brain and peripheral tissues in depression across the life span: a review of replicated findings. Neurosci Biobehav Rev. 2016;71:281–93.
29. Corkum CP, Ings DP, Burgess C, Karwowska S, Kroll W, Michalak TI. Immune cell subsets and their gene expression profiles from human PBMC isolated by Vacutainer Cell Preparation Tube (CPT) and standard density gradient. BMC Immunol. 2015;16:48.
30. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA. 2001;98:5116–21.
31. Plaisier SB, Taschereau R, Wong JA, Graeber TG. Rank-rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures. Nucleic Acids Res. 2010;38:e169.
32. Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol. 2005;4:Article17.
33. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinform. 2008;9:559.
34. Langfelder P, Luo R, Oldham MC, Horvath S. Is my network module preserved and reproducible? PLoS Comput Biol. 2011;7:e1001057.
35. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102:15545–50.
36. Korotkevich G, Sukhov V, Sergushichev A. Fast gene set enrichment analysis. bioRxiv. 2019:060012.
37. Arloth J, Bogdan R, Weber P, Frishman G, Menke A, Wagner KV, et al. Genetic differences in the immediate transcriptome response to stress predict risk-related brain function and psychiatric disorders. Neuron. 2015;86:1189–202.
38. Breen MS, Bierer LM, Daskalakis NP, Bader HN, Makotkine I, Chattopadhyay M, et al. Differential transcriptional response following glucocorticoid activation in cultured blood immune cells: a novel approach to PTSD biomarker development. Transl Psychiatry. 2019;9:201.
39. Kramer A, Green J, Pollard J Jr, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014;30:523–30.
40. Daskalakis NP, Cohen H, Cai G, Buxbaum JD, Yehuda R. Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. Proc Natl Acad Sci USA. 2014;111:13529–34.
41. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. 2014.
42. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. Sociol Methodol. 1982;13:290–312.
43. Mascha EJ, Dalton JE, Kurz A, Saager L. Statistical grand rounds: understanding the mechanism: mediation analysis in randomized and nonrandomized studies. Anesth Analg. 2013;117:980–94.
44. Bliese P. Multilevel modeling in R (2.5).
45. Houtepen LC, Vinkers CH, Carrillo-Roa T, Hiemstra M, van Lier PA, Meeus W, et al. Genome-wide DNA methylation levels and altered cortisol stress reactivity following childhood trauma in humans. Nat Commun. 2016;7:10967.
46. Oster H, Challet E, Ott V, Arvat E, de Kloet ER, Dijk DJ, et al. The functional and clinical significance of the 24-hour rhythm of circulating glucocorticoids. Endocr Rev. 2017;38:3–45.
47. Chan JC, Nugent BM, Bale TL. Parental advisory: maternal and paternal stress can impact offspring neurodevelopment. Biol Psychiatry. 2018;83:886–94.
48. Bowers ME, Yehuda R. Intergenerational transmission of stress in humans. Neuropsychopharmacology. 2016;41:232–44.
49. Tobi EW, Goeman JJ, Monajemi R, Gu H, Putter H, Zhang Y, et al. DNA methylation signatures link prenatal famine exposure to growth and metabolism. Nat Commun. 2014;5:5592.
50. Peter CJ, Fischer LK, Kundakovic M, Garg P, Jakovcevski M, Dincer A, et al. DNA methylation signatures of early childhood malnutrition associated with impairments in attention and cognition. Biol Psychiatry. 2016;80:765–74.
51. Daskalakis NP, Yehuda R. Site-specific methylation changes in the glucocorticoid receptor exon 1F promoter in relation to life adversity: systematic review of contributing factors. Front Neurosci. 2014;8:369.
52. Jablonska-Trypuc A, Matejczyk M, Rosochacki S. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem. 2016;31(sup1):177–83.
53. Gajendrareddy PK, Engeland CG, Junges R, Horan MP, Rojas IG, Marucha PT. MMP-8 overexpression and persistence of neutrophils relate to stress-impaired healing and poor collagen architecture in mice. Brain Behav Immun. 2013;28:44–8.
54. Loffek S, Schilling O, Franzke CW. Series "matrix metalloproteinases in lung health and disease": biological role of matrix metalloproteinases: a critical balance. Eur Respir J. 2011;38:191–208.
55. Rodgers AB, Morgan CP, Bronson SL, Revello S, Bale TL. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. J Neurosci. 2013;33:9003–12.
56. Tobi EW, Slieker RC, Luijk R, Dekkers KF, Stein AD, Xu KM, et al. DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. Sci Adv. 2018;4:eaao4364.
57. Monk C, Spicer J, Champagne FA. Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways. Dev Psychopathol. 2012;24:1361–76.
58. Morgan CP, Bale TL. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. J Neurosci. 2011;31:11748–55.
59. Schwaiger M, Grinberg M, Moser D, Zang JC, Heinrichs M, Hengstler JG, et al. Altered stress-induced regulation of genes in monocytes in adults with a history of childhood adversity. Neuropsychopharmacology. 2016;41:2530–40.