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Differential DNA Methylation is associated with Hippocampal Abnormalities in Pediatric Posttraumatic Stress Disorder

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Abstract

Background: Recent findings in neuroimaging and epigenetics offer important insights into brain structures and biological pathways of altered gene expression associated with posttraumatic

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stress disorder (PTSD). However, it is unknown to what extent epigenetic mechanisms are associated with PTSD and its neurobiology in youth.

Methods: In this study we combined a methylome-wide association study and structural neuroimaging measures in a Dutch cohort of youth with PTSD (ages 8-18 years). We aimed to replicate findings in a similar independent American cohort.

Results: We found significant methylome-wide associations for pediatric PTSD (FDR $p < 0.05$) compared to non-PTSD control groups (traumatized and non-traumatized youth). Methylation differences on 9 genes were replicated, including genes related to glucocorticoid functioning. In both cohorts, methylation on OLFM3 gene was further associated with anterior hippocampal volume.

Conclusions: These findings point to molecular pathways involved in inflammation, stress response, and neuroplasticity as potential contributors to neural abnormalities and provide potentially unique biomarkers and treatment targets for pediatric PTSD.

Keywords

Posttraumatic Stress Disorder; epigenetics; Methylation; imaging; child and adolescent psychiatry; biological pathways

Introduction

Childhood trauma is common and imposes a substantial psychological burden on youth. Approximately two-thirds of youth are exposed to psychological trauma before they reach adulthood, and up to 16% of all youth develop post-traumatic stress disorder (PTSD) by the age of 18 (1, 2). Youth suffering with PTSD often show lower academic achievement than their non-affected counterparts; this decrease in achievement is coupled with increased rates of depression, suicide attempts, and substance abuse into adulthood (3, 4). These statistics highlight the need for additional research into the neurodevelopmental underpinnings of pediatric PTSD, with the goals of improving detection, prognosis, and treatment.

The developmental origins of health and disease hypothesis posits that early life exposures, including exposure to childhood trauma, will have prolonged effects on child health, including neurodevelopmental outcomes. Epigenetic alterations provoked by exposure to childhood trauma are likely to play a central role in the molecular mechanisms underlying the emergence of PTSD in youth. Specifically, trauma-related alterations in DNA methylation (DNAm) at cytosine-guanine junctions (CpGs) may influence the developmental programming of neural circuitry underlying stress responses, including the hippocampus, amygdala, and medial prefrontal cortex (mPFC) (5).

Important strides have been made in identifying epigenetic substrates of adult PTSD using peripheral DNAm markers (6-8). Other studies have also reported associations between DNAm and brain phenotypes in predominantly healthy cohorts (9-12). However, despite these promising results, it remains unclear whether similar DNAm markers are present in a pediatric PTSD, which is of critical importance given ongoing physiological and neurodevelopment in youth. It is also unclear whether these DNAm markers are related to

neural abnormalities previously identified in pediatric PTSD, which is important to identify potential molecular/cellular pathways associated with altered neurodevelopment contributing to pediatric PTSD (13). In addition, previous studies examining childhood trauma are mostly based on *a priori* searches of candidate genes(14). However, as is common with a priori searches, this approach increases the risk of confirmation biases, thereby increasing the risk of false negatives for useful epigenetic biomarkers of developmental psychopathology after adversity.

To address these knowledge gaps, we performed a Methylome-wide association study (MWAS) using a discovery cohort of youth with PTSD, compared with two control groups. The first group (Dutch sample) consisted of trauma-exposed youth without PTSD (a resilient group), and a second group of healthy non traumatized youth. We then attempted to replicate these DNAm findings in an independent (American) cohort of youth with PTSD and a non-traumatized comparison group.

In addition to our MWAS, we investigated whether altered methylation was further related to brain structure in regions involved in emotion regulation. This combined approach has two major advantages. First, the use of independent cohorts for discovery and replication of DNAm findings should reduce the likelihood of false positive findings and enhance generalizability of results. Second, identifying which peripherally-derived methylation differences are linked to structural brain differences in trauma-exposed and PTSD youth may point to genes with the most biological relevance for pediatric trauma and PTSD through their link to central nervous system abnormalities. Given prior work implicating altered methylation in glucocorticoid pathway genes in childhood trauma and adult PTSD, we hypothesized that youth with PTSD would show altered methylation in genes annotated to the HPA axis, which would then be further associated with prefrontal and hippocampal gray matter volume abnormalities previously identified in pediatric PTSD (15-19). We aimed to minimize DNAm findings that were either likely to occur by chance (by requiring replication) and that may not have an impact on neural systems (by requiring association with gray matter volume). Complementing these specific hypotheses, we aimed to identify novel methylation abnormalities and methylation-brain relationships in pediatric PTSD through our MWAS.

METHODS

Participants

In the present study, two independent MWAS were performed. The first cohort (N=224) was recruited at the Specialized Centre for Trauma and Family Treatment of the Bascule/ Department of Child and Adolescent Psychiatry of the Amsterdam University Medical Center(The Netherlands). The second cohort (N=44) was recruited from area mental health facilities in Madison, Wisconsin, USA. In both cohorts, medication-free youth with PTSD were included (Partial or Full PTSD diagnosis on CAPS-CA interview). They were matched for age and sex with non-traumatized, typically-developing healthy comparison (NTC) youth. In the Dutch cohort an additional control group of traumatized comparison youth without PTSD (TC) was recruited. These cohorts have been previously described (20, 21)with additional information in supplementary methods and materials. All procedures

were approved by the Medical Ethical Committee of the University Medical Center and the University of Wisconsin Health Sciences institutional review board.

Clinical and Behavioral Assessments

Assessments in these cohorts have been previously described. Briefly, participants and their caregivers completed structured psychiatric and trauma screening including the CAPS-CA. Additional self- and parent-report of youth symptoms of depression and anxiety were obtained using standardized questionnaires. Please see supplementary methods and materials for additional detail. To facilitate comparisons between study samples, Table 1 provides the rates of children that score above clinical cut-off of internalizing and externalizing symptoms based on these questionnaires.

DNA Acquisition and Extraction and generation of methylation signal

In both cohorts three milliliters of saliva was collected and stored in Oragene DNA sample collection kits (DNA Genotek, Canada). DNA was extracted using a Gentra autopure LS system following manufacturers protocol. For additional detail about the bisulfite conversion, see supplementary file. Methylation signal was generated using the HumanMethylation EPIC/850 BeadChip (Dutch cohort) and 450 BeadChip (USA cohort) following the manufacturer's guidelines. See for details, supplementary file.

Quality Control and Data Processing

In both cohorts, prior to hybridization, cases and controls were randomized across the 96 well plates. Technical replicates (n = 8) were included for quality control of array, monitoring potential batch effects. Identical analysis pipelines were implemented across cohorts using R (v.3.4.2). In the supplementary file, each step is briefly described, followed by the R package and functions used during implementation. According to these steps, plate batch, ethnicity, sex and age were selected to be included as covariates in the statistical model. Ethnicity was based on genetic information from 27523 SNPs, thus we decided to keep these SNPs for genetic information, however without the X and Y genes, and cross hybridization probes. The second component of our prinicipal component analysis (PCA2) correlated with ethnicity (.03). We therefore added PCA2 as a covariate in our analysis. Next, we identified group-related differentially methylated positions (DMPs) and regions (DMRs), using a general linear model (*lmfit*) that accounts for the main effects between groups: (1) PTSD vs NTC in both cohorts and (2) PTSD vs TC in the Dutch cohort. To correct for inflation of p-values the BACON package was used (22), the Lambda's after using BACON are reported in Supplementary Table 6. A false discovery rate (FDR) for DMPs and a family wise error rate (FWER) for DMRs was used to adjust for multiple testing ($p. < .05$). These corrections were applied to the results from the Dutch correct, but not the American cohort. Because the American cohort was used as a replication sample, stringent corrections would risk unduly and artificially increasing the rate of false negative findings. Therefore in the American cohort a Bonferroni correction ($p < 0.007$) was applied.The Bonferroni threshold was the by-product of dividing critical alpha = 0.05 by the number of GOIs identified in the Dutch cohort. Bonferroni corrections were combined with Fisher's method for combining p values from the independent tests across CpGs to approximate the DMR p-value ($p. <0.5$) (23, 24).DMRs were analyzed with use of the

Minfi function *bumphunter*. DMRs were defined to include at least 2 probes in the cluster. For each significantly associated DMR beta-values of each of the individual CpGs were extracted, which were used in subsequent post-hoc and sensitivity analyses. CpGs were visualized using the R package *coMET (v.1.14.0)* (25). We used M-values for the analysis (also for calculating the Bacon P values), and Beta values for visualizing our data in the figures.

MRI Acquisition

For the Dutch cohort all scans were acquired using a 3.0T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) equipped with a SENSE eight-channel receiver head coil. For each participant, a T1-weighted structural MRI image was acquired with the following parameters: TE: 3.527 ms, TR: 9 ms, slice thickness: 1 mm, 170 slices, flip angle: 8○ and image matrix 256 x 256 that covert the entire brain. For the American cohort, MRI acquisition parameters are similar and have been reported elsewhere (Keding & Herringa 2015).

Image Preprocessing and Voxel-Based Morphometry

The Dutch and American cohorts used the same image preprocessing and voxel-based morphometry (VBM) procedures, which have been reported elsewhere (26), see supplement for additional information. Gray matter volume (GMV) from previously identified regions of interest in youth with PTSD (ROIs - hippocampus, mPFC, fusiform gyrus) (21) were extracted for focused comparisons with DNA methylation. See supplemental methods and materials for additional information.

Post Hoc Analyses

Peak DNA methylation at DMPs and DMRs and their associations with GMV were assessed using partial correlation and regression analyses in R (*ppcor*) in a subset of the Dutch cohort (see Supplementary Table 4) and in the entire American cohort. Due to regulatory restrictions in the Dutch cohort it was not possible to collect neuroimaging in the non-traumatized control group.

We selected one significant DMP (significant in the Dutch cohort after multiple testing correction) and nine DMRs that showed an overlap within the two cohorts for our post-hoc analysis. MWAS replication analysis was defined as overlap for at least one DMP within a DMR for both cohorts, comparing the top 1000 DMRs. For post hoc analysis we performed both partial correlation analyses between GMV available for each cohort and the information from the original DMR. Analyses were covaried for age, sex, trauma type and PTSD duration. Additionally, these results were further corrected for multiple comparisons using an FDR correction and a Bonferroni correction for the number of post-hoc analyses conducted. Post-hoc results with a corrected $p < 0.007$ (7 sets of partial correlation analyses) were considered statistically significant.

RESULTS

Demographic and mental health measures of participating youth are shown in Table 1 and 2 and further described in supplement.

EWAS analysis

A set of MWAS (differentially-methylated regions [DMRs] and differentially-methylated positions [DMPs]) contrasting the PTSD and comparison groups identified statistically significant CpG sites. DMR analysis comparing the groups identified significant CpG regions at FWER < 0.05. In the Dutch cohort comparing PTSD with the NTC, the most significant effect was reported on the *TNXB* gene (FWER = 0.03) and the *PM20D1* gene (FWER = 0.04). At the *TNXB* gene PTSD youth showed hyper methylation compared to NTC, at the *PM20D1* gene PTSD youth showed hypomethylation compared to NTC. The list of the top 10 CpG regions in both cohorts are provided in Supplementary Table 1 and 2. The findings annotated to the *TNXB* and *PM20D1* gene (PTSD vs. NTC youth) were replicated with the same set of MWAS in the American cohort. Results are shown in Table 3.

In the Dutch cohort, the most significant DMP was on the corticotrophin-releasing hormone binding protein (*CRHBP*) gene, located at cg26196496 (FDR < 0.02). Here, PTSD youth showed hyper methylation at this CpG relative to NTC youth. A plot showing greater detail on the CpGs in or near *CRHBP* is provided in Supplementary Figure 1. Furthermore, PTSD youth showed hypomethylation at a CpG site located on cg21972431 relative to trauma comparison (TC) youth (FDR < 0.01). These DMP findings did not replicate in the American cohort. The lists of the top 5 CpG sites, derived from the DMP analysis in both cohorts, are shown in Supplementary Table 3 and 4..

Exploratory Analysis of DMR/DMP Conjunction in the Dutch and American Cohorts

Next, we compared the top 1000 DMRs between the two cohorts to assess potentially important DNAm abnormalities not detected by the more stringent multiple comparison correction threshold in the Dutch cohort EWAS analysis. This revealed nine DMRs that were associated with PTSD (relative to NTC youth) in both cohorts. These DMRs are shown in Table 3. In both cohorts, we identified overlap on two very large DMRs showing hypermethylation in PTSD youth relative to NTC youth (63 CpGs in the Dutch cohort, 68 CpGs in the American cohort) annotated on the *TNXB* gene. An overview of the DMR outcomes per cohort are shown in Supplementary Table 1. The DMP conjunction analysis did not reveal overlap between the two cohorts. The results of the DMP analyses by cohort are reported in Supplementary Table 3 and 4..

Associations Between DNA Methylation and Gray Matter Volume

We next assessed potential relationships between DNAm findings with gray matter volume in regions of interest (ROIs) previously identified in pediatric PTSD including the ventromedial prefrontal cortex, hippocampus, and amygdala (13). We performed partial correlations to associate regional grey matter volume with peak methylation on the DMPs/DMRs that survived our multiple testing correction in the Dutch cohort or that were

replicated in the American cohort. Only a subgroup of the youth participated in this post hoc analyses (see online methods).

These analyses revealed that a DMR annotated to Olfactomedin 3 (*OLFM3*) showed a significant negative correlation with right anterior hippocampus GMV in both cohorts (Supplementary Figure 2). Additional findings only detected in one cohort are shown in Table 4 and Supplementary Figure 2.

All results presented above remained significant after sensitivity analyses controlling for trauma-related variables, suggesting the difference is specific to the development of pediatric psychopathology and not a consequence of trauma type, time elapsed since the trauma, and trauma load. For an overview of the outcomes see Table 4.

DISCUSSION

To our knowledge, this is the first study in which biological pathways of altered gene expression and brain structures implicated in pediatric PTSD have been investigated. Using two independent, international cohorts, the results of this study confirm for the first time the hypothesis that pediatric PTSD is associated with epigenetic modifications which, in turn, are associated with structural neurophenotypes implicated in pediatric PTSD. Most notably, altered DNA methylation on genes annotated to *PM20D1, TNXB* and *OLFM3* genes were replicated across cohorts and exhibited a relationship to neural structure in at least one of the cohorts. Because this is the first genome wide methylation study in youth with PTSD to date, we cannot compare these results with other cohorts (15). It is notable, however, that our results do not overlap with recent findings in adult PTSD (Smith, Ratanatharathorn (6). This is perhaps not surprising, given that the current study focused on a developmental sample, in which trauma load, comorbidities, substance use, and many other factors markedly differ from adult PTSD samples. However, our findings do thematically overlap with studies of childhood trauma and adult PTSD, suggesting abnormal methylation in genes annotated to the inflammation and stress response systems (15).

First, changes observed on *OLFM3* gene were related to hippocampal volume in both cohorts. This may represent an interaction between methylation and neuronal change related to early exposure to traumatic events and the development of PTSD at an early stage in life. OLFM3 is a neuronal protein, found throughout the brain and related to the development of microglia (27, 28). Microglia serve as key immune cells of the brain, and are activated in response to signals they receive or detect in their microenvironment. For example, they respond to injuries and infections and are able to modify the structure and function of a cell. They can for example lead to neuronal degeneration, impaired microglia activation has been linked to the development of brain disorders, such as neurodegenerative diseases (29). Epigenetic mechanisms have emerged as important regulators in this process (30). In addition to their essential role in the development of the central nervous system (CNS), microglial dysfunction is suggested to be involved in stress vulnerability and depression recurrence (31). These findings are in line with our results that suggest that hyper methylation of OLFM3 may be a molecular mechanism by which hippocampal volume

is decreased in youth with PTSD, potentially via enhanced CNS inflammation in affected youth.

Secondly, methylation changes in the *TNXB* gene were observed in both cohorts. *TNXB* encodes an extracellular-matrix associated glycoprotein that has been implicated in cell migration processes (ProteinAtlas), and was shown to be hypermethylated in PTSD youth relative to NTC youth. Furthermore, an additional significant effect was reported comparing PTSD with NTC youth in the Dutch cohort. This suggests that there is an effect specific to pediatric PTSD (and/or its comorbid disorders) rather than trauma-exposure generally. We also observed that in the American cohort across PTSD youth and NTC youth, *TNXB* methylation is negatively associated with gray matter volume in the anterior hippocampus, originally identified in a group by age interaction in Keding & Herringa (2015). This relationship was not observed in the Dutch cohort, however this could be due to the missing NTC in the MRI group. Interestingly, *TNXB* has been shown to have protein-protein interactions with the protein products of *VEGFA*, *VEGFB*, *NEURL1*, and *NEURL 1B* (https://string-db.org/), all four of which have been heavily implicated in cellular processes in the anterior hippocampus. More specifically, these proteins are functionally related to hippocampal-dependent synaptic plasticity, learning, and memory (https://www.genecards.org/). This suggests that methylation of *TNXB* may act as a molecular mechanism of altered synaptic plasticity or growth affecting hippocampal volume in pediatric PTSD, which may then have implication for multiple hippocampal functions such as context discrimination, fear and extinction learning, and emotion regulation implicated in pediatric PTSD (13).

In addition to the results of the DMR analysis, we detected DNAm abnormalities on the *CRHBP* gene in the Dutch cohort. This gene is known for its critical role in the regulation of corticotrophin-releasing hormone binding protein (CRH-BP), which in turn plays a modulatory role in the function of the HPA axis as well as CRH signaling within the brain. The HPA-axis is thought to play an important role in the etiology of PTSD, and the regulation of emotions and behavior (15). In addition, numerous studies in rodents have shown that levels of CRH-BP in various brain regions are highly responsive to psychological stressors (32). Furthermore, studies comparing post mortem brain tissue found that altered CRH-BP expression has been associated with adult psychopathology and suicide completion (33, 34). In the case of pediatric PTSD, it is possible that altered CRH-BP methylation, and thus altered expression, may contribute to abnormal CRH signalizing both peripherally and centrally, contributing to heightened physiological and stress responses associated with PTSD. In line with earlier studies, these findings further support the involvement of abnormal gene regulation associated with HPA axis dysregulation.

While this study highlights potentially novel genetic and molecular mechanisms contributing to pediatric PTSD, several limitations should be acknowledged. First, we used data generated on different arrays between the two cohorts (HumanMethylation450 in the Dutch cohort and EPIC bead chip array in the US cohort). Data generated with these arrays are comparable (the EPIC bead chip covers the whole 450k array), but only capture a fraction of the CpG sites in the genome. Second, this study has a limited sample size in both cohorts, creating risk for both false negative and false positive findings particularly in a genetic study.

To mitigate the latter, we included youth with carefully diagnosed, phenotypes to increase the probability that important, and true, group differences would be identified. However, replication is a strength that in part helps to overcome the small sample size. Another limitation regarding our cohorts are the comorbid problems that have been reported, such as ADHD and depression which may limit specificity of findings to PTSD.

Additionally, we applied strict corrections in the Dutch (MWAS)search, with replication in the American cohort at an uncorrected level, in order to reduce the overall risk of false positive results while balancing the risk of false negatives. Third, our study examined gene methylation using only saliva samples. Though this approach likely captures part of the underlying pathophysiology of pediatric PTSD, it is unlikely to represent exact DNA methylation in brain regions most relevant for PTSD. To mitigate this possibility, we have emphasized DNAm abnormalities that also map onto known structural brain abnormalities in pediatric PTSD.

Future studies would be warranted to collect longitudinal data in youth with and without trauma-exposure and PTSD to determine the temporal course of methylation-neural structure relationships in relation to childhood trauma exposure and the emergence of PTSD. Given the implication of inflammatory and glucocorticoid pathways in this initial study, future studies would also benefit from including other peripheral or central markers such as cortisol and cytokine levels, and determine whether these represent mediating factors between altered peripheral methylation and structural brain changes leading to pediatric PTSD. Finally, future studies would benefit from collecting additional tissue sources (blood, saliva and eventually post-mortem brain tissue), as well as information about RNA and protein expression.

In conclusion, our findings provide new insights into the underlying biology and potential biomarkers for PTSD in youth. To our knowledge, this is the first investigation into differential DNA methylation in pediatric PTSD and its association to structural brain abnormalities. While youth with PTSD are an inherently difficult population to recruit, we utilized a combination of two independent, international, and well-phenotyped cohorts, a common data analytic pipeline, strict statistical corrections, and linkage to neural phenotypes, to increase the likelihood of important and reproducible findings in pediatric PTSD. These findings offer future targets for hypothesis driven studies in larger samples and statistical rigor for early identification of candidate gene pathways. If replicated in subsequent work, the epigenetic markers identified here could serve as novel therapeutic targets in the prevention and treatment of pediatric PTSD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Demographic information, trauma history and clinical characteristics in the Dutch cohort

	PTSD youth (n=74)	Traumatized Controls (n=75)	Healthy non-traumatized
Sex			
Boys	31 (41.9 %)	37 (49.3 %)	36 (48.0 %)
Girls	43 (58.1 %)	38 (50.7 %)	39 (52.0 %)
Age	12.13 (3.44)	11.95 (2.28)	10.77 (2.15)^{a**}
Ethnicity			
Caucasian	44 (59.5 %)	61 (81.3 %) ^{c**}	
Other	30 (40.5 %)	14 (18.7 %)	64 (85.3 %)^{c**}
Index trauma			
Interpersonal violence	38 (54.4%)	21 (35.6%)	X
Sexual abuse	11 (14.9%)	0 (0%)	X
Accidents/medical	9 (12.2%)	37 (49.3%)	X
Comorbid diagnosis			
Internalizing problems	23 (31.1 %)	2 (2.7 %)^{c**}	0 (0 %)^{c**}
Externalizing problems	18 (24.3 %)	3 (4.0 %)^{c**}	0 (0 %)^{c**}
CAPS-CA Severity Score	52.91 (26.07)	14.45 (12.88)^{b**}	X
CRIES-13 Severity Score	37.44 (15.35)	11.04 (15.07)^{b**}	X

Continuous variables presented as mean (standard deviation); categorical variables presented as frequency (percentage).

^{a}= One-way ANOVA;

^{b}= Kruskal-Wallis Test;

^{c}= Chi-Square Test.

**significant group differences (p > .05). PTSD=post-traumatic stress disorder; CAPS-CA = Clinician Administered PTSD Scale for Children and Adolescents; CRIES-13 = Children's revised Impact of Event Scale, Revised. Comorbid internalizing and externalizing symptoms were based on the Revised Children's Anxiety and Depression Scale (RCADS; T- scores ≥70), and the Child Behavioral Checklist (CBCL) and Youth Self Report (YSR; T score ≥63).

Table 2:

Demographic Information, Trauma History and Clinical Characteristics in the USA cohort

	PTSD Youth (n=22)	Healthy non-traumatized
Sex		
Boys	8	7
Girls	14	13
Age	14.22 (2.70)	14.21 (2.80)
Ethnicity		
Caucasian	18	17
Other	4	3
IQ	103.2 (13.0)	110.5 (12.5)
Left Handed	0	0
Index Trauma		
Interpersonal Violence	3 (13.6%)	-
Sexual Abuse	11 (50%)	-
Severe	3 (13.6%)	-
Accident/medical trauma Other (Traumatic News, Natural Disaster)	5 (22.7%)	-
Comorbid Diagnoses		
Depression	19 (86.4%)	-
Anxiety	14 (63.6%)	-
ADHD	6 (27.3%)	-
Previous Psychotropic Medication-Use		
Depression	8 (36.4%)	-
Anxiety	3 (13.6%)	-
ADHD	7 (31.8%)	-
CAPS-CA Severity Score	77.11 (16.75)	-
PTSD-RI Severity Score	55.36 (11.14)	-

Continuous variables presented as mean (standard deviation); categorical variables presented as frequency (percentage). There were no significant group differences (PTSD Youth vs. Healthy Youth; $p > 0.05$) in sex, ethnicity, or handedness distribution, age, or IQ (based on χ^2 tests of independence and independent samples *t*-tests respectively). PTSD = Post-Traumatic Stress Disorder; ADHD = Attention Deficit, Hyperactivity Disorder; CAPS-CA = Clinician-Administered PTSD Scale for Children and Adolescents; PTSD-RI = UCLA PTSD Reaction Index. Comorbid diagnosis were based on the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS), Mood and Feelings Questionnaire (MFQ), and Screen for Child Anxiety Related Emotional Disorders (SCARED).

Table 3:

Overlapping DMRs identified in the PTSD vs Non-Traumatized Control group in both cohorts.

	Gene	Chr	*p*-value Dutch Cohort	FWER Dutch Cohort	*p*-value USA Cohort	p-value Fisher's method	Direction
PTSD Youth vs. NTC Youth	*TNXB*	6	< 0.001	0.030	0.00033	0.02334**	PTSD > NTC
	PM20D1	1	< 0.001	0.040	6.58E-05	0.01104**	NTC > PTSD
	TNXB	6	< 0.001	0.100	0.00169	0.0992	PTSD > NTC
	DUSP22	6	< 0.001	0.320	0.00119	0.13888	NTC > PTSD
	GDF7	2	0.002	0.958	0.00119	0.415772	NTC > PTSD
	SLC1A4	2	0.004	0.990	0.00550	0.99	NTC > PTSD
	KLHL35	11	0.004	0.998	0.00018	0.596804	PTSD > NTC
	ZNF714	19	0.009	1	0.00206	0.996	NTC > PTSD
	OLFM3	1	0.016	1	0.02906	1	PTSD > NTC

Detected DMRs (L>2) using minfi's "bumphunter" function; comparison based on the top 1000 hits in both cohorts. All findings met threshold of a combined FWER calculated with Fisher's method of < 0.05.

** indicates a significant result in both cohorts. DMR = Differently methylated regions, Chr = Chromosome; PTSD = Post-Traumatic Stress Disorder; NTC = Non-Traumatized Control; FWER = Family-Wise Error Rate.

Table 4:

Significant post-hoc partial correlation results comparing peak methylation on DMPs/DMRs with regional grey matter volume estimates

	Gene	Chr	Region	*r* Dutch Cohort	*p*-value Dutch Cohort	FDR *p*-value Dutch Cohort	*r* USA Cohort	*p*-value USA Cohort	FDR *p*-value USA Cohort
PTSD Youth and NTC Youth	*CRHBP*	5	R Fusiform Gyrus	0.328	0.002	0.018		-	-
	CRHBP	5	L Fusiform Gyrus	0.350	< 0.001	0.016		-	-
	GDF7	2	R Anterior Hippocampus	0.288	0.006	0.037		-	-
	GDF7	2	R vmPFC	0.294	0.005	0.037		-	-
	GDF7	2	R Fusiform Gyrus	-	-	-	0.455	< 0.001	0.003
	GDF7	2	L Fusiform Gyrus	-	-	-	0.375	0.003	0.018
	OLFM3	1	R Anterior Hippocampus	−0.323	0.002	0.018**	−0.453	< 0.001	0.003**
	OLFM3	1	R vmPFC	−0.369	< 0.001	0.014	-	-	-
	PM20D1	1	R Fusiform Gyrus	-	-	-	0.384	0.003	0.017
	SLC1A4	2	R Fusiform Gyrus	-	-	-	0.511	< 0.001	0.001
	SLC1A4	2	L Fusiform Gyrus	-	-	-	0.335	0.010	0.043
	TNXB	6	R Anterior Hippocampus	-	-	-	−0.406	0.001	0.012

Partial correlations were controlled for age and sex of participant. All findings met threshold of FDR *p*-value < 0.05.

** indicates a significant result in both cohorts; Chr = Chromosome; DMR = Differently methylated regions; DMP =Differently methylated position; PTSD = Post-Traumatic Stress Disorder; NTC = Non-Traumatized Control; vmPFC = ventromedial prefrontal cortex

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use “this paper” if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Commercial Assay Or Kit	Human: Saliva	DNA Genotek, Canada		
Other	EPIC/850 BeadChip	Illumina, Inc.		
Other	450 BeadChip	Illumina, Inc.		
Other	EZ DNA Methylation-Gold kit	Zymo Research		
Other	Qubit	Qiagen, USA		
Software; Algorithm	R version 3.5.0	R Project for Statistical Computing	RRID:SCR_001905	
Software; Algorithm	Methylaid package	R Project for Statistical Computing	RRID:SCR_002659	
Software; Algorithm	Minfi package	R Project for Statistical Computing	RRID:SCR_012830	
Software; Algorithm	CoMet package	R Project for Statistical Computing	RRID:SCR:011925	
Software; Algorithm	Statistical Parametric Mapping	Wellcome Department of Imaging Neuroscience, London, UK	RRID:SCR:007037	
Software; Algorithm	VBM8 toolbox	http://dbm.neuro.uni-jena.de/vbm/)		
Software; Algorithm	MATLAB v9.1	Mathworks	RRID:SCR_001622	

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