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DNA methylation from germline cells in veterans with PTSD

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ABSTRACT

In this study we investigated genome-wide sperm DNA methylation patterns in trauma-exposed Vietnam veterans. At the genome-wide level, we identified 3 CpG sites associated with PTSD in sperm including two intergenic and one CpG within the *CCDC88C* gene. Of those associated with PTSD in sperm at a nominal level, 1868 CpGs were also associated with PTSD in peripheral blood (5.6% overlap) including the *RORA, CRHR1* and *DOCK2* genes that have been previously implicated in PTSD. A total of 10 CpG sites were significantly associated with a reported history of a diagnosed mental health condition in children and reached genome-wide significance. CpGs associated with a history of a reported mental health condition in children were also enriched (90% of tested genes) for genes previously reported to be resistant to demethylation, making them strong candidates for transgenerational inheritance. In conclusion, our findings identify a unique sperm-specific DNA methylation pattern that is associated with PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) is a disabling mental health condition that can develop after exposure to trauma, characterised by symptoms such as mood changes, intrusive memories, hyperarousal, and avoidance behaviour. Epidemiological studies estimate that about 75% of the population will experience at least one traumatic event during their lifetime (Breslau and Kessler, 2001), yet only a subset of individuals go on to develop PTSD. Several risk factors have been identified for the development of PTSD, which include socioeconomic and sociodemographic factors such as education level, employment, and marital status, in addition to a family history of psychiatric disorders and childhood trauma (Breslau, 1999; Breslau and Davis, 1992; Brewin et al., 2000). More recently, there has been increasing interest in identifying genetic and epigenetic risk factors involved in the development of, and resilience to PTSD (Bruenig et al., 2017a, 2017b; Mehta et al., 2016); and in particular, epigenetic risk factors that can be both inherited, and altered by environment. Parental lifetime stress exposures, likely transferred to children via epigenetic marks in germ cells, can contribute to the programming of subsequent generations' stress axis development and reactivity, factors critical in individual disease vulnerability to PTSD (Rodgers and Bale, 2015).

One of the most common and widely studied epigenetic mechanisms is DNA methylation, which can alter gene expression. DNA methylation

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involves the addition of a methyl group at cytosine–phosphate-guanine dinucleotide (CpG) sites in the genome. DNA methylation has been associated with psychosocial stress, childhood trauma, depression and PTSD (Klengel et al., 2014). Our previous studies have identified DNA methylation differences in PTSD that are associated with a history of childhood abuse (Klengel et al., 2013; Mehta et al., 2013). Recently, our team performed a genome-wide study in combat-exposed veterans and we identified novel genes that showed peripheral blood DNA methylation differences in PTSD (Mehta et al., 2017). Following on from this study we also showed that some of these DNA methylation markers were associated with differential gene expression (Mehta et al., 2018). Since DNA methylation changes accumulate over time, the molecular signature of trauma exposure may be evident substantially before symptoms of a disorder develop.

The concept of intergenerational transmission of trauma is still debatable and while some studies in animals have shown this phenomenon to exist (Dias and Ressler, 2014) there is currently not much evidence from human studies (Yehuda and Lehrner, 2018). The earliest evidence of the transgenerational effects of parental lifetime stress experience comes from an animal study in which exposure to stress in adult female rats before mating altered offspring behavioural stress responses across two subsequent generations (Wehmer et al., 1970). In humans, children of parents with PTSD have been shown to have higher rates of lifetime PTSD, evidence of a family 'ripple effect' which has also been termed 'secondary traumatisation' (Yehuda, 2004). Children of Jewish parents with PTSD who experienced the Holocaust showed lower basal plasma cortisol levels and elevated rates of PTSD (Yehuda et al., 2007). Another study found that children of Vietnam veteran fathers had an increased risk of PTSD of 5-fold for sons and 3-fold for daughters (O'Toole et al., 2017).

There is accumulating evidence suggesting the transgenerational transmission of DNA methylation changes from parents to children. Exposure to stress can cause disruption in the hypothalamic pituitary adrenal (HPA) axis, hence, genetic studies have primarily focused on genes involved in this system. In one study, Perroud et al. investigated epigenetic changes associated with PTSD in children of women who were pregnant at the time of the Tutsi genocide in Rwanda (Perroud et al., 2014). The authors found that both mothers exposed to genocide related trauma and their children had significantly higher rates of PTSD and depression as well as higher DNA methylation levels at exon 1F in the promoter region of the glucocorticoid receptor gene (*NR3C1*) compared to controls. Moreover, the *NR3C1* methylation was negatively correlated with glucocorticoid levels in plasma. Yehuda et al. (2014a) also demonstrated that in the absence of maternal PTSD, children with paternal PTSD showed higher DNA methylation of the exon 1F promoter of the glucocorticoid receptor, while children with both maternal and paternal PTSD showed lower methylation. This demonstrated that paternal PTSD effects were moderated by maternal PTSD effects. Lower glucocorticoid receptor (GR) exon 1F promoter methylation was significantly associated with greater post-dexamethasone cortisol suppression. In a subsequent study Yehuda and colleagues examined transgenerational peripheral DNA methylation changes in a gene that regulates GR sensitivity (*FKBP5*) in Holocaust survivors (Yehuda et al., 2016). This study identified significantly higher *FKBP5* intron 7 DNA methylation in Holocaust survivors and significantly lower *FKBP5* intron 7 DNA methylation in their children. The authors suggested that this observed opposite effect might be attributable to biological accommodation in the children. These findings suggest that trauma-induced DNA methylation changes can be transmitted from parents to children, however these studies were limited by their small sample sizes and the fact that the DNA methylation was performed in peripheral blood cells.

The mechanisms of transgenerational transmission of epigenetic signatures are complex. Inheritance of epigenetics is not 100%, as 'epigenetic reprogramming' of germ line cells can occur via demethylation, however, some genes are resistant to this (Franklin et al., 2010; Gapp et al., 2014; Morgan and Bale, 2011). A recent key paper investigating fertility markers using whole genome methylation arrays identified 94 genes that appear to be resistant to demethylation during epigenetic reprogramming (Camprubi et al., 2017). The study revealed spermatozoa contain a profile that is normally hypomethylated but a select number of sites maintain a hypermethylated state. These sites are likely to be important in transgenerational epigenetic inheritance.

To date, no study has interrogated DNA methylation differences in germline cells of veterans with PTSD. The aim of this pilot study was to perform a genome-wide scan of the human sperm epigenome to:

a) Compare differential tissue-specific DNA methylation patterns in peripheral blood and semen in veterans with PTSD and depressive symptoms
b) Investigate candidate genes and genome-wide DNA methylation patterns in semen samples of veterans with and without PTSD
c) Test the association between fathers' psychiatric phenotypes and DNA methylation in semen with a reported history of a mental health condition in their now adult children

2. Materials and methods

Samples: A total of 299 male veterans who had served in the Australian or New Zealand armed services in Vietnam during the Vietnam War were recruited between February 2014 and July 2015 by a specialised veteran mental health unit at Greenslopes Private Hospital (GPH) in Brisbane through the Gallipoli Medical Research Foundation (GMRF) and Returned and Services League of Australia (RSL) websites, through RSL publications and newspaper and television advertisements, and by word of mouth (McLeay et al., 2017). Of these, 159 participants met criteria for PTSD diagnosis and the remaining 140 participants were assigned to the control group. Participants were provided with a sample cup for semen prior to their scheduled blood collection date and asked to return it at their next visit. All veterans provided blood samples and a subset (n = 38) of veterans provided semen samples. For the blood DNA methylation, we selected veterans with current PTSD diagnosis based on CAPS-5 (n = 48) and veterans with no current and no previous PTSD diagnosis (n = 48) as cases and controls respectively.

A total of 104 samples (38 semen and 96 peripheral blood) were included in this study for DNA methylation, with paired samples available for 30 participants (semen and peripheral blood). Semen samples used in the analysis were collected within at average of 12 days of blood collection for DNA extraction and analysis." The semen samples included 22 veterans with no PTSD and 16 veterans with PTSD. This study was approved by the Greenslopes Human Ethics Committee, Department of Veterans Affairs Human Research Ethics Committee, and Queensland University of Technology (QUT) Human Research Ethics Committee. All participants provided written informed consent.

Clinical Assessments: Clinical and life experience data, including psychiatric and physical health diagnoses and combat-trauma exposure were available for these veterans (McLeay et al., 2017) and interview-based data was supplemented by pre-military and military data from Army records, including information about combat exposure. Structured clinical history included demographics and information on smoking, diet and exercise habits, lifetime history of alcohol consumption, past and current illnesses and medications. Structured military and combat history information including term of service, number of times served, role and duration in the defence force was also collected. All clinical assessments were completed within six weeks of screening and collection of the biological samples.

PTSD symptom severity was assessed using the Clinician Administered PTSD Scale for DSM-5 (CAPS-5) (Weathers et al., 2014). PTSD diagnosis was performed using the CAPS-5 and the diagnosis was validated through structured interviews by psychiatrists. Common comorbidities were assessed using the Mini International Neuropsychiatric Interview DSM IV (MINI), an instrument designed to assess major

Axis 1 disorders with high validity and reliability (Lecrubier et al., 1997; Sheehan et al., 1997). The Depression Anxiety Stress Scale 21 (DASS-21) is a self-report scale that measures through subscales three different constructs: stress, depression and anxiety (Lovibond and Lovibond, 1995). The Cronbach's Alpha DASS-21 was high with an $\alpha = 0.95$. The Patient Health Questionnaire (PHQ-9) was used in addition to screen for depression. The self-rating instrument for depression PHQ-9 was developed from the Primary Care Evaluation of Mental Disorders (PRIME-MD) (Spitzer et al., 1999) and based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for MDD. The Connor-Davidson Resilience Scale (CD RISC) was used to measure resilience via a range of coping strategies that have been shown to be successful in dealing with adversity (Connor and Davidson, 2003). The scale has good psychometric properties (Bezdjian et al., 2016), with a Cronbach's $\alpha = 0.93$. Mental health diagnoses of adult children were determined by a clinician-administered questionnaire asking the veterans if any of their children had ever been diagnosed with a mental health condition (yes/no/unsure/prefer not to answer/not applicable), followed by a free text section to name the condition(s) and provide further details. For analysis, data were coded as yes (one or more children diagnosed with a mental health condition) or no (no diagnosis).

Experimental Procedures: The semen samples were frozen in sterile specimen jars at −80 °C until processing. DNA extraction was performed on 1 mL aliquots of thawed semen. Each semen sample was centrifuged at high speed to separate the cellular fraction from the seminal plasma (14000 rpm for 20 min) and the pellet was resuspended in 200 μL of lysis buffer (20 mM Tris·HCl, pH 8.0, 20 mM EDTA, 200 mM NaCl, 4% SDS, 80 mM DTT and 12.5 μL/mL proteinase K) and incubated at 56 °C for a minimum of 60 min. The samples were vortexed twice during incubation to disperse the homogenate. When the pellet was completely dissolved, 200 μL Buffer AL (Qiagen, Chadstone, Australia) was added to the sample. The suspension was vortexed and incubated at 70 °C until the precipitate dissolved. The remainder of the DNA extraction and purification was performed using the Qiagen QiAMP Mini DNA extraction kit as per the manufacturer's instruction. Extracted DNA was initially assessed for quality and quantity using the Nanodrop spectrophotometer (Thermo Fisher Scientific, Brendale, Australia). DNA samples were stored frozen at −80 °C until transfer to the Australian Genome Research Facility (AGRF) for epigenetic analysis.

Blood samples were sent to AGRF for DNA extraction as previously described (Mehta et al., 2017). Genomic DNA was extracted from a 2 mL blood sample, using MACHERY-NAGEL NucleoSpin L (MACHEREY-NAGEL GmbH & Co. KG, Dueren, NRW, Germany).

Quality assessment of the sperm and blood DNA samples was performed by resolution on a 0.8% agarose gel at 130 V for 60 min and Quanti-iT (Thermo Fisher Scientific, Australia) fluorescence assessment. Samples were bisulphite converted (500 ng) with Zymo EZ DNA Methylation kit (Mehta et al., 2013; Wockner et al., 2014). The microarray experimental procedures have previously been described (Mehta et al., 2017).

Statistical analysis: Raw beta values from EPIC Illumina arrays were exported into R (Version 3.4.0, The R Foundation for Statistical Computing) for statistical analysis. The level of methylation was determined by calculating a "β value" (the ratio of the fluorescent signals for the methylated *vs*. unmethylated sites). The raw data was background and control-normalized using the Bioconductor MINFI package (1.4.0) (Aryee et al., 2014).

Data were analysed using an established analysis pipeline comprising of custom statistical programs and scripts (Barfield et al., 2014; Mehta et al., 2011, 2013) written in R and Linux. Surrogate variable analyses (SVA) of the sperm DNA methylation revealed two significant SVA vectors which were used as covariates in the model to correct for technical artefacts and hidden confounds (Leek and Storey, 2007). We have previously used this method to correct for ethnicity, cell counts, current medication, BMI and smoking (Mehta et al., 2017). Generalized regression models were performed to identify genomic sites significantly associated with PTSD, where the DNA methylation betas were regressed against the PTSD symptom severity and the SVAs were used as covariates. For association with reported children mental health status, DNA methylation betas were regressed against the reported children mental health status and the SVAs were used as covariates. Results were corrected for multiple testing using a 10% false discovery rate (FDR).

Functional annotation of the genes corresponding to the top 100 CpG sites was performed using the KEGG database via the Webgestalt interface (Wang et al., 2013) to identify enriched pathways with at least three genes within the pathway using a hypergeometric test for enrichment evaluation analysis and adjusted p-value of less than 0.05 after Bonferroni correction.

3. Results

3.1. Demographics of samples

Of the 104 veterans, 53 veterans had current PTSD and 51 veterans had no current PTSD. The average age was 68.44 [0.42] years. Demographics and characteristics of the 38 veterans with sperm DNA methylation and the complete 104 veteran cohort are shown in Table 1 and Supplementary Table 1 respectively.

3.2. PTSD-associated DNA methylation in sperm versus peripheral blood

We interrogated DNA methylation patterns associated with PTSD in semen samples from veterans (n = 38) and tested their overlap with previously reported DNA methylation patterns associated with PTSD in peripheral blood from veterans (n = 96). Sample demographics are provided in Supplementary Table 1. Of these, a total of 30 veterans were included in both sperm and peripheral blood analyses. Of 33,129 CpG sites associated with PTSD in sperm ($p < 0.05$), 1,868 CpGs (1,176 unique genes) were also associated with PTSD in peripheral blood (5.6% overlap, enrichment p-value > 0.05, Supplementary Table 2 and Supplementary Fig. 1). Functional annotation via the KEGG database revealed that the overlapping genes were enriched for RNA transport, cell adhesion molecules, metabolic pathways and pathways in cancer (adjusted p-value < 0.05). Among the genes overlapping between sperm and peripheral blood were genes previously reported to be associated with PTSD including *RORA, DOCK2* and *CRHR1*.

3.3. PTSD-associated DNA methylation differences in sperm

To identify DNA methylation patterns associated with PTSD in semen samples from veterans (n = 16 with PTSD, 22 without PTSD), sperm DNA methylation was tested for association with PTSD symptom severity after correcting for SVAs.

We specifically tested two genes, the glucocorticoid receptor gene (*NR3C1/GR*) and its co-chaperone the *FKBP5* gene whose DNA methylation have previously been reported to be transgenerationally transmitted from parents to children (Perroud et al., 2014; Yehuda et al., 2014b, 2016). We tested a total of 41 CpGs within the *FKBP5* gene and a total of 89 CpGs within the *GR* gene that were present in the dataset. DNA methylation of a total of four CpGs (two CpGs within *GR* and two CpGs within *FKBP5*) were significantly associated with PTSD in the veterans ($p < 0.05$) but none of these survived multiple testing correction (Table 2).

At the genome-wide level, a total of three CpG sites were significantly associated with PTSD symptom severity after correction for multiple testing (Table 3 and Fig. 1). These included two intergenic CpG sites and one CpG within the *CCDC88C* gene. Interestingly, two CpG sites within the *CCDC88C* gene were also associated with PTSD in peripheral blood (Supplementary Table 2).

Table 1
Demographics of the 38 veterans with sperm DNA methylation.

Phenotype	Mean [SE]/N [%]		P-value
	Non PTSD	PTSD	Group difference
	n = 22	n = 16	
Service type: Army	14	14	0.251
Airforce	6	1	
Navy	2	1	
Age (in years)	67.77 [0.67]	67.00 [0.58]	0.409
Berlin BMI	28.99 [0.72]	28.96 [0.85]	0.983
Marital status: Married	18 [81.8%]	13 [81.3%]	0.569
Divorced	2 [9.1%]	2 [12.5%]	
Other (Single/Widowed)	2 [9.1%]	1 [6.3%]	
Employment status: Retired	14 [63.6%]	5 [31.3%	0.012
Full-time working	4 [18.2%]	0 [0%]	
Part-time working	1 [4.5%]	2 [12.5%]	
Not working	3 [13.6%]	9 [56.3%]	
PTSD Symptom Severity score (CAPS)	2.68 [0.73]	16.38 [1.94]	1.03×10^{-8}
PTSD Symptom Severity score (CAPS)	75.55 [2.8]	72.63 [4.54]	0.568
PTSD Symptom Severity score (CAPS)	2.14 [0.45]	6.13 [1.37]	0.003
PTSD Symptom Severity score (CAPS)	1.18 [0.38]	6.00 [1.21]	0.00012
PTSD Symptom Severity score (CAPS)	4.73 [0.78]	10.56 [1.41]	0.00045
PTSD Symptom Severity score (CAPS)	2.50 [0.57]	8.31 [1.30]	6.6×10^{-5}
PTSD Symptom Severity score (CAPS)	5 [22.7%]	5 [31.3%]	0.556
PTSD Symptom Severity score (CAPS)	1 [4.5%]	1 [4.5%]	0.671
PTSD Symptom Severity score (CAPS)	0 [0%]	1 [4.5%]	0.475
PTSD Symptom Severity score (CAPS)	0 [0%]	2 [12.5%]	0.088
PTSD Symptom Severity score (CAPS)	1 [4.5%]	9 [64.3%]	0.00031

Functional annotation via the KEGG database revealed that the top 100 CpGs were significantly enriched for genes within the TGF-beta signalling (adjusted p-value = 0.0025), focal adhesion (adjusted p-value = 0.027) and regulation of actin cytoskeleton (adjusted p-value = 0.0320) pathways.

3.4. Association of paternal phenotypes and sperm DNA methylation with mental health in veterans 'children

We tested the association of psychiatric phenotypes in the veteran with a reported mental health condition in one or more of their children. Of the 38 veterans with sperm DNA methylation available, 32 had children and 6 did not - 9 veterans had one child, 12 veterans had 2 children, 8 had 3 children, 2 veterans had 4 children and 1 veteran had 6 children. Hence a total of 71 offspring (25 males, 36 females and 10 unknown) were analysed. The PTSD symptom severity in veterans was not significantly associated with history of a mental health condition in their children (p = 0.061) however a trend for significance was observed. The DASS-21 depression (p = 0.005), anxiety (p = 0.014) and stress (p = 0.014) scores in veterans significantly predicted reported presence of a mental health diagnosis in their children. Additionally, the PHQ9 scores in the veterans were also significantly associated with reported mental health diagnosis in their children (p = 0.014).

To investigate the likelihood of paternal transmission of DNA methylation from the father to their children, we tested if veteran sperm DNA methylation patterns were associated with a reported diagnosed mental health condition in one or more of their children. For the *GR* and *FKBP5* candidate genes, DNA methylation of a total of 20 CpG sites (16 in *GR* and 4 in *FKBP5*) was associated with mental health diagnosis in their children ($p < 0.05$) and of these one CpG in *GR* and one CpG in *FKBP5* survived correction for multiple testing (Table 2). At the genome-wide level a total of 10 CpG sites were significantly associated with the presence of a reported mental health condition in their children after correction for multiple testing. The CpGs were located within the *LCP1, SKOR1, PAX6, CUX1, GLTSCR1, CCDC85C, VWDE, DCLK2 and PLEKHG5* genes (Table 3 and Fig. 2). For eight of these nine genes (*LCP1, PAX6, CUX1, GLTSCR1, CCDC85C, DCLK2, PLEKHG5* and *VWDE*), methylation in at least one CpG site was also associated with PTSD in sperm DNA as well as peripheral blood DNA (Supplementary Table 2).

Next, we compared the results to a previously reported list of 94 genes that were shown to be resistant to demethylation in sperm, making them strong candidates for transgenerational inheritance (Camprubi et al., 2017). Of the 94 candidate genes, a total of 69 genes were present on the DNA methylation array and were interrogated. A total of 62 out of the 69 (90% of tested genes) transgenerational inheritance candidate genes also showed significant sperm DNA methylation differences in veterans associated with a reported mental health condition in one or more of their adult children (Supplementary Table 3).

Functional annotation via the KEGG database revealed that the top 100 CpGs were significantly enriched for genes within the insulin signalling pathway (adjusted p-value = 7.38e-5).

4. Discussion

Comparison of PTSD associated DNA methylation marks in sperm versus those in peripheral tissues may help identify genes and pathways involved in the development of PTSD, and provide insight into the heritability of PTSD via epigenetic changes. In this novel study we performed a genome-wide scan of the sperm epigenome to compare DNA methylation differences among veterans with and without PTSD.

Three major analyses were performed, one to test the overlap of PTSD-associated DNA methylation in sperm and peripheral blood; one to identify DNA methylation patterns in sperm associated with PTSD and another to test fathers' psychiatric phenotypes and DNA methylation patterns in sperm associated with a reported mental health condition in one or more of their children.

At the genome-wide level, three CpGs were significantly associated with PTSD symptom severity after correction for multiple testing including two intergenic CpG sites and one CpG within the *CCDC88C* gene that encodes a ubiquitously expressed coiled-coil domain-containing protein that interacts with the dishevelled protein and is a negative regulator of the *Wnt* signalling pathway. Previously, mutations within the *CCDC88C* gene have been associated with congenital hydrocephalus (Drielsma et al., 2012; Ruggeri et al., 2018), different types

Table 2
CpG sites in *GR* and *FKBP5* genes associated with PTSD and reported diagnosis of mental health condition in adult children.

CpG	Gene	Pvalue PTSD	Beta Effect PTSD	Pvalue child mental health	Beta Effect child mental health
cg00052684	FKBP5	7.36E-01	2.65E-04	2.60E-01	−1.74E-02
cg00130530	FKBP5	2.54E-01	3.40E-04	5.52E-01	3.54E-03
cg00140191	FKBP5	2.25E-01	1.94E-04	2.18E-01	3.90E-03
cg00610228	FKBP5	2.97E-01	−1.09E-04	1.11E-03	−6.29E-03
cg00862770	FKBP5	6.63E-01	−4.18E-05	3.28E-01	1.85E-03
cg01294490	FKBP5	6.20E-02	2.21E-04	9.01E-01	3.00E-04
cg03098337	FKBP5	1.15E-01	−7.13E-04	9.19E-01	9.26E-04
cg03245912	FKBP5	5.75E-01	9.09E-05	3.00E-01	−3.32E-03
cg03546163	FKBP5	2.08E-01	−1.59E-03	9.28E-02	−4.17E-02
cg03591753	FKBP5	5.95E-01	2.12E-04	2.61E-01	−8.82E-03
cg04137760	FKBP5	3.71E-01	3.92E-04	3.32E-01	8.44E-03
cg04791658	FKBP5	1.98E-01	4.60E-04	3.82E-01	6.24E-03
cg05039098	FKBP5	4.47E-01	8.05E-04	2.15E-01	−2.59E-02
cg06087101	FKBP5	9.37E-01	4.96E-05	1.30E-01	−1.86E-02
cg06409316	FKBP5	5.99E-01	2.94E-04	2.27E-01	1.33E-02
cg06937024	FKBP5	5.47E-01	−5.36E-05	9.09E-02	−2.93E-03
cg07061368	FKBP5	1.41E-02	−1.68E-03	9.45E-01	−9.77E-04
cg07485685	FKBP5	4.01E-02	−4.28E-04	9.33E-02	−7.01E-03
cg07633853	FKBP5	7.67E-01	−3.10E-04	8.93E-03	−5.16E-02
cg07696519	FKBP5	9.69E-01	3.59E-05	2.32E-01	−2.17E-02
cg07843056	FKBP5	6.84E-01	−5.90E-05	3.35E-01	−2.76E-03
cg08586216	FKBP5	4.04E-01	−2.23E-04	1.02E-01	−8.56E-03
cg08636224	FKBP5	3.27E-01	9.08E-04	2.73E-02	3.94E-02
cg09268536	FKBP5	4.19E-01	−5.65E-04	3.06E-02	−2.91E-02
cg11845071	FKBP5	3.82E-01	−1.03E-04	2.28E-01	−2.82E-03
cg11905112	FKBP5	1.82E-01	−1.20E-03	3.73E-01	−1.59E-02
cg13344434	FKBP5	9.10E-01	−6.91E-05	4.87E-01	−8.37E-03
cg14284211	FKBP5	5.93E-01	−5.81E-04	5.56E-02	−4.02E-02
cg14339974	FKBP5	4.46E-01	−8.33E-04	7.97E-01	−5.60E-03
cg14642437	FKBP5	8.05E-01	−1.98E-04	5.80E-02	2.94E-02
cg15929276	FKBP5	9.96E-01	4.26E-06	4.91E-01	−1.04E-02
cg16052510	FKBP5	7.80E-01	3.15E-04	7.36E-01	7.55E-03
cg16912838	FKBP5	2.67E-01	−8.30E-04	4.38E-01	−1.16E-02
cg17030679	FKBP5	4.20E-01	1.40E-04	6.75E-01	−1.44E-03
cg17085721	FKBP5	9.75E-02	−1.16E-03	6.23E-01	−6.95E-03
cg19014730	FKBP5	6.59E-01	5.17E-04	5.32E-01	1.45E-02
cg19226017	FKBP5	6.16E-01	−3.39E-04	3.33E-01	−1.29E-02
cg20813374	FKBP5	4.10E-01	−2.46E-04	7.27E-01	−2.07E-03
cg22363520	FKBP5	1.17E-01	8.10E-04	5.53E-01	−6.17E-03
cg23416081	FKBP5	2.10E-01	1.48E-03	2.63E-01	2.63E-02
cg24295963	FKBP5	8.38E-01	−1.58E-04	4.59E-01	−1.13E-02
cg00294552	NR3C1	7.06E-02	−1.14E-03	7.28E-01	4.43E-03
cg00407401	NR3C1	6.55E-01	−3.47E-04	4.03E-01	−1.29E-02
cg00629244	NR3C1	4.12E-01	9.48E-05	1.64E-01	3.16E-03
cg01294526	NR3C1	7.19E-02	−9.83E-04	5.71E-01	−6.28E-03
cg01751279	NR3C1	4.92E-01	4.84E-04	4.09E-01	−1.15E-02
cg01967637	NR3C1	6.35E-01	−9.14E-05	8.64E-02	−6.41E-03
cg03746860	NR3C1	3.93E-01	−5.16E-04	9.13E-01	−1.31E-03
cg03857453	NR3C1	4.58E-01	−4.42E-04	1.15E-01	−1.84E-02
cg03906910	NR3C1	8.26E-01	−2.69E-04	4.55E-02	−4.72E-02
cg04111177	NR3C1	5.98E-01	−5.03E-05	7.22E-01	−6.75E-04
cg04457787	NR3C1	8.37E-01	1.32E-04	3.16E-01	−1.27E-02
cg05483455	NR3C1	6.11E-02	−9.23E-04	3.81E-03	−2.73E-02
cg05900547	NR3C1	6.79E-01	−4.20E-04	3.24E-01	−1.98E-02
cg06521673	NR3C1	8.47E-01	−1.15E-05	3.20E-01	−1.16E-03
cg06613263	NR3C1	3.87E-01	8.20E-04	2.00E-01	2.39E-02
cg06952416	NR3C1	7.12E-01	1.32E-04	8.97E-01	−9.14E-04
cg06968181	NR3C1	6.42E-01	−1.00E-04	1.48E-01	−6.10E-03
cg07515400	NR3C1	9.43E-01	1.11E-05	3.70E-01	−2.74E-03
cg07528216	NR3C1	7.72E-01	9.95E-05	1.21E-01	−1.04E-02
cg07589972	NR3C1	7.76E-01	1.72E-04	2.27E-01	−1.43E-02
cg07715663	NR3C1	5.32E-01	3.82E-04	8.10E-01	2.93E-03
cg07733851	NR3C1	3.21E-01	4.03E-04	5.57E-03	2.13E-02
cg07742588	NR3C1	3.32E-01	−7.75E-04	2.63E-01	1.77E-02
cg08423118	NR3C1	4.14E-01	6.80E-04	6.46E-01	−7.61E-03
cg08695103	NR3C1	7.63E-01	−2.60E-04	6.20E-02	−3.11E-02
cg08818984	NR3C1	2.08E-01	−2.15E-03	6.50E-03	−8.85E-02
cg08845721	NR3C1	4.05E-01	5.77E-04	8.12E-03	3.47E-02
cg10847032	NR3C1	6.13E-01	−6.80E-05	6.23E-01	−1.32E-03
cg11152298	NR3C1	3.86E-01	6.40E-05	1.20E-01	−2.25E-03
cg12466613	NR3C1	3.58E-01	−4.79E-04	2.02E-01	−1.31E-02
cg12741214	NR3C1	1.09E-01	−6.59E-04	4.89E-02	−1.59E-02
cg12888360	NR3C1	7.49E-01	3.43E-04	5.48E-01	−1.27E-02
cg12969488	NR3C1	9.99E-02	8.75E-04	1.79E-03	3.13E-02
cg13514002	NR3C1	7.37E-01	−1.52E-04	6.54E-01	−4.03E-03
cg13648501	NR3C1	4.54E-01	9.37E-05	7.46E-01	−8.08E-04
cg13764763	NR3C1	7.92E-01	1.42E-04	1.01E-01	1.72E-02
cg14438279	NR3C1	9.45E-01	−4.17E-05	3.79E-01	1.04E-02
cg14558428	NR3C1	6.87E-01	4.71E-05	5.59E-01	1.36E-03
cg14621978	NR3C1	1.99E-02	−1.34E-03	2.75E-01	−1.29E-02
cg14939152	NR3C1	7.88E-01	−2.55E-05	2.95E-02	−3.96E-03
cg15115787	NR3C1	9.93E-01	5.01E-06	6.80E-01	4.84E-03
cg15645634	NR3C1	1.83E-01	−1.18E-04	3.44E-01	−1.67E-03
cg15910486	NR3C1	2.33E-01	−2.00E-04	2.55E-01	−3.80E-03
cg16219186	NR3C1	3.50E-01	6.50E-04	5.06E-03	3.68E-02
cg16224829	NR3C1	1.62E-01	−6.72E-04	5.02E-02	−1.84E-02
cg16335926	NR3C1	7.02E-01	−3.32E-05	8.28E-01	3.74E-04
cg16535116	NR3C1	3.64E-01	−8.64E-04	5.94E-01	−1.01E-02
cg16586394	NR3C1	1.89E-01	−5.40E-04	8.61E-01	−1.45E-03
cg16594263	NR3C1	2.36E-02	−1.47E-03	3.18E-01	−1.32E-02
cg17342132	NR3C1	4.24E-01	5.05E-04	5.72E-03	3.29E-02
cg17349736	NR3C1	9.30E-01	−5.40E-05	6.77E-01	5.06E-03
cg17617527	NR3C1	6.35E-01	−3.94E-05	8.82E-01	2.45E-04
cg17860381	NR3C1	2.39E-01	−1.05E-04	4.51E-01	−1.34E-03
cg18019515	NR3C1	3.62E-01	−6.82E-05	4.21E-02	−2.95E-03
cg18068240	NR3C1	1.80E-01	−1.32E-04	4.43E-01	−1.51E-03
cg18146873	NR3C1	9.09E-01	−1.88E-05	9.81E-01	−7.81e-05
cg18484679	NR3C1	8.24E-01	1.50E-04	2.30E-02	−2.94E-02
cg18718518	NR3C1	1.13E-01	4.82E-04	7.95E-005	2.17E-02
cg18849621	NR3C1	3.46E-01	1.15E-04	1.76E-01	3.25E-03
cg18998365	NR3C1	3.57E-01	3.75E-04	8.93E-03	2.02E-02
cg19135245	NR3C1	1.01E-01	4.14E-04	3.19E-01	5.07E-03
cg19176661	NR3C1	6.12E-01	−2.94E-04	3.16E-01	1.14E-02
cg19432243	NR3C1	9.49E-01	6.68E-05	7.15E-01	7.55E-03
cg19457823	NR3C1	4.64E-01	−6.29E-04	8.58E-01	3.06E-03
cg19645279	NR3C1	9.45E-01	6.96E-05	2.48E-01	2.29E-02
cg19820298	NR3C1	9.90E-01	1.23E-05	3.22E-01	1.84E-02
cg20598211	NR3C1	9.91E-01	−8.33E-06	4.18E-01	−1.15E-02
cg20728768	NR3C1	8.16E-02	−1.34E-03	4.61E-01	−1.15E-02
cg20753294	NR3C1	5.75E-01	−1.68E-04	5.47E-02	−1.11E-02
cg21209684	NR3C1	5.34E-01	6.73E-05	6.45E-01	9.90E-04
cg21702128	NR3C1	5.03E-01	−6.11E-05	2.57E-01	−2.03E-03
cg21979215	NR3C1	7.00E-01	4.60E-04	6.60E-01	−1.04E-02
cg22233604	NR3C1	3.37E-01	4.17E-04	8.02E-01	2.18E-03
cg22402730	NR3C1	6.38E-01	3.97E-05	7.09E-01	−6.25E-04
cg23273257	NR3C1	9.42E-01	−2.58E-05	9.53E-02	−1.16E-02
cg23430507	NR3C1	5.35E-01	4.32E-04	2.57E-02	2.98E-02
cg23776787	NR3C1	4.74E-01	−9.27E-04	8.32E-02	−4.37E-02
cg24026230	NR3C1	8.20E-01	3.68E-05	8.41E-01	6.44E-04
cg24052866	NR3C1	5.83E-01	−3.75E-04	6.68E-01	5.83E-03
cg24801588	NR3C1	8.52E-01	−1.55E-04	1.15E-01	2.56E-02
cg25535999	NR3C1	6.24E-01	−3.19E-04	4.58E-01	−9.56E-03
cg25579735	NR3C1	6.11E-01	2.91E-04	1.15E-01	1.76E-02
cg25708981	NR3C1	9.83E-01	−2.26E-05	5.57E-01	1.27E-02
cg26081259	NR3C1	2.39E-02	−1.83E-03	9.78E-01	−4.64E-04
cg26464411	NR3C1	3.07E-01	2.76E-04	2.81E-01	5.77E-03
cg26720913	NR3C1	5.55E-01	−1.02E-03	3.99E-02	−6.86E-02
cg27107893	NR3C1	5.79E-01	−1.29E-03	1.02E-01	−7.41E-02
cg27122725	NR3C1	8.79E-01	5.14E-05	7.22E-01	2.38E-03
cg27345592	NR3C1	9.57E-01	5.11E-05	9.27E-02	3.09E-02

Beta Effect = the beta effect of the regression of CpGs associated with PTSD and child mental health.

of cancer (Ara et al., 2016; Gosenca et al., 2014) and spinocerebellar ataxia (Tsoi et al., 2014). To the best of our knowledge there is no link of the *CCDC88C* gene to stress-related disorders however in a mouse model, 5-hydroxymethylcytosine modification of CCDC88C was reported to be associated with exposure to acute stress (Li et al., 2016). Comparison with CpGs that were significantly associated with PTSD symptom severity in peripheral blood showed a non-significant overlap

Table 3a
CpGs associated with PTSD severity.

CpG	P-value	P-value children mental health	Chr	Base pair	Beta effect	Gene symbol
cg10076395	5.58E-07	6.11E-01	7	140101434	0.0027	*SLC37A3* (intergenic)
cg08786732	7.88E-07	1.33E-01	13	40765955	−0.0032	*SLC25A15* (intergenic)
cg23701703	3.37E-06	1.43E-01	14	223947	−0.0017	*CCDC88C*

(5.6%, 1,176 genes), indicating that these DNA methylation marks were sperm-specific. This included genes previously reported to be associated with PTSD including *RORA* and *CRHR1*. Additionally, of the 1176 genes associated with PTSD in both sperm and peripheral blood, 145 genes were also associated with a reported mental health condition in adult children. These genes are of interest given that they were reported to be significantly associated with PTSD in both analyses and are more likely to represent genes whose DNA methylation might be transgenerationally transmitted via the germline. Among these genes was the *DOCK2* gene which we have previously reported to be associated with PTSD (Mehta et al., 2017).

Functional annotation of the differentially methylated sites via the KEGG database revealed that the top 100 CpGs associated with PTSD symptom severity were overrepresented for genes within the TGF-beta signalling, focal adhesion and regulation of actin cytoskeleton pathways. A study of mice lacking TGF-beta signalling in dopaminergic neurons supports a role for TGF-beta in regulating dopaminergic and GABAergic neurons and contribution to psychiatric disorders (Luo et al., 2016).

For association with a reported history of diagnosed mental health condition in adult children, at the genome-wide level a total of 10 CpG sites were significant after correction for multiple testing. The CpGs were located within the *LCP1, SKOR1, PAX6, CUX1, GLTSCR1, CCDC85C, VWDE, DCLK2* and *PLEKHG5* genes. All of these genes show previous association with psychiatric disorders. Of the 10 CpGs, the CpG within the *VWDE* gene was also associated with PTSD symptom severity in the veterans in sperm while for eight of the nine genes, at least one CpG was associated with PTSD in both sperm and peripheral blood. *VWDE* (von Willebrand factor D and EGF domains) is a gene worth following up as high levels of von Willebrand factor is a recognised risk factor for coronary heart disease. Increased levels of von Willebrand factor antigen is also associated with PTSD and may explain the increased risk of thrombosis and cardiovascular complaints in PTSD patients (Robicsek et al., 2011). The *LCP1* is the L-plastin gene whose phosphorylation is important for immune synapse maturation. It has been previously demonstrated that glucocorticoid dexamethasone inhibits L-plastin phosphorylation, thereby preventing maturation of the immune synapse (Wabnitz et al., 2011). Another interesting gene was the *CUX1* that plays a specific role in the development of cortical pyramidal neurons (Li et al., 2010). There is previous evidence that polymorphisms of the *CUX1* gene may be associated with response to antidepressant treatment in patients with MDD (Sasayama et al., 2013). *SKOR1* or *MAP2K5* is a gene identified in GWASs to be associated with

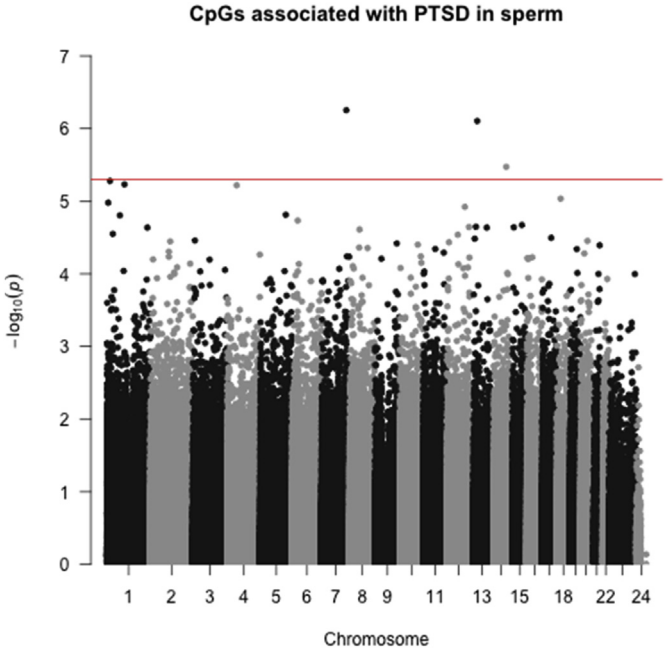

Fig. 1. Manhattan plot of CpG sites associated with PTSD in sperm samples from veterans. The red line indicates CpG sites significant at 10% false discovery rate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

restless leg syndrome which is often comorbid with anxiety and depression disorders (Catoire et al., 2018). *PAX6* methylation significantly predicts depressive symptomatology in children (Kaufman et al., 2018) and plays a key role in neurogenesis and differentiation of dopaminergic neurons (de Chevigny et al., 2012). *PLEKHGS* is involved in neuronal cell differentiation and is associated with motor neuron disease (Maystadt et al., 2007). *PLEKHGS* differential methylation is also associated with depression in men who have been exposed to early life stress (Khulan et al., 2014). *DCLK2* is expressed in the brain and double DCLK2 and DXC (microtubule-associated protein) knockout mice have severe abnormalities in the hippocampus (Hayashi et al., 2015). *GLTSCR1* is likely to play a role as a calcium channel modulator as it was identified as an expression quantitative trait locus for *SLC8A2* which is a known calcium exchanger in resting state arousal (Jawinski et al., 2018). One of the major symptoms of PTSD is hypervigilance and

Table 3b
CpGs associated with children mental health diagnosis.

CpG	P-value	P-value PTSD	Chr	Base pair	Beta effect	Gene symbol
cg06477663	4.97E-07	4.01E-01	13	46757415	−0.0393	*LCP1*
cg11923198	7.37E-07	2.72E-01	15	68125277	0.0096	*SKOR1*
cg06705930	1.22E-06	1.75E-01	11	31823191	0.1181	*PAX6*
cg10541043	2.42E-06	1.94E-01	7	101892633	0.0404	*CUX1*
cg15889872	3.18E-06	4.73E-01	11	31823181	0.0496	*PAX6*
cg11589723	3.34E-06	3.36E-01	19	48205483	0.0474	*GLTSCR1*
cg12773397	3.40E-06	3.23E-01	14	100066736	−0.0436	*CCDC85C*
cg03579179	4.29E-06	2.55E-02	7	12444095	0.0213	*VWDE*
cg14311957	4.46E-06	8.20E-02	4	151130601	−0.0331	*DCLK2*
cg16146806	4.75E-06	8.56E-01	1	6530205	0.0526	*PLEKHG5*

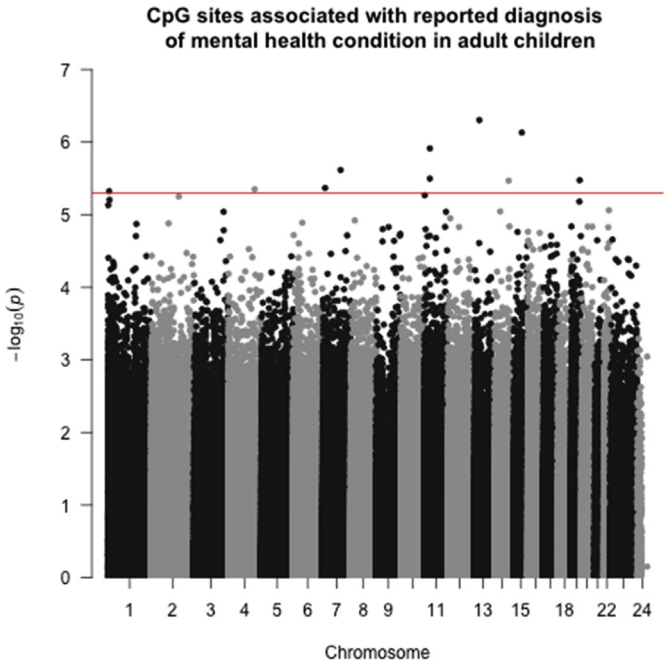

Fig. 2. Manhattan plot of CpG sites associated with reported diagnosis of mental health condition in adult children. The red line indicates CpG sites significant at 10% false discovery rate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

arousal. *CCDC85C* is associated with cortical development and differential methylation of *CCDC85C* is associated with early childhood adversity (Yang et al., 2013).

While PTSD symptom severity showed a trend for association, the DASS 21 depression, anxiety and stress scores in veterans significantly predicted reported mental health diagnosis in their children, suggesting that paternal psychiatric symptoms were associated with the mental health status of their children. We tested DNA methylation patterns associated with PTSD by focussing on the *GR* and *FKBP5* genes previously reported to be transgenerationally transmitted from parents to children in DNA methylation studies in humans (Perroud et al., 2014; Yehuda et al., 2016). DNA methylation of a total of 4 CpGs (2 CpGs in *GR* and 2 CpGs in *FKBP5*) were significantly associated with PTSD in the veterans ($p < 0.05$) but none of these survived multiple testing corrections. However, testing the association of these genes with a reported mental health condition in their adult children revealed 20 significant CpG sites (16 in *GR* and 4 in *FKBP5*) and one CpG in *GR* and one CpG in *FKBP5* survived correction for multiple testing. It is worthwhile noting that the previous studies performed on *GR* and *FKBP5* genes were candidate-based studies performed in peripheral blood samples hence were likely to be missed in a genome-wide analysis as they would be unlikely to survive multiple testing correction. Previous studies (Perroud et al., 2014; Yehuda et al., 2016) have shown than *GR* and *FKBP5* genes DNA methylation patterns are transmitted from parents to offspring and these are associated with mental health, hence we hypothesize that the association with offspring mental health likely reflects underling inherited DNA methylation differences in these genes that influence the stress sensitivity and regulate the HPA neuroendocrine stress axis.

Of note, sperm DNA methylation differences in veterans who reported a mental health condition in their adult children were also enriched (90% of tested genes) for genes previously reported to be resistant to demethylation in sperm, making them strong candidates for transgenerational inheritance (Camprubi et al., 2017). Functional annotation revealed an overrepresentation of genes within the insulin signalling pathway. This is of interest given the link between PTSD and metabolic disorders (Konjevod et al., 2019). The insulin signalling pathway was also significantly enriched in a genome-wide methylation study of PTSD in world trade centre first-responders (Kuan et al., 2017).

Our study has several limitations. This is a small study and replication of these findings in larger samples is warranted. This study has focussed on DNA methylation as a likely mechanism for transgenerational transmission of trauma and PTSD marks. However, there are alternate mechanisms of transgenerational transmission apart from experience-dependent changes in parental behaviour such as exposure to environmental toxins. Smoking for example has been associated with epigenetic changes in mature sperm, suggesting that molecular signatures in germ cells in addition to parental behaviour may be poised to pass on information about the parental environment to their children (Li et al., 2012; Marczylo et al., 2012). Also, DNA methylation can be affected by several different factors and while we have attempted to account for these, it is likely that there are other unaccounted covariates that remain to be addressed. Furthermore, we were unable to test directly for transgenerational transmission as we did not have access to DNA methylation patterns in the children of veterans, nor did we formally evaluate mental health histories of veterans' children; hence we used fathers' reports of a diagnosis of mental health conditions in their children as a surrogate measure. We recognise that these self-reports may be confounded by the psychological state or diagnosis of the participant and factors such as residing with the children and family support might also influence the results. We acknowledge that in addition to putative biological mechanisms many other risk factors may increase the risk for PTSD incidence in military personnel. Analysis of the biological pathways of differentially methylated genes was performed at a gene level, hence there might be a bias towards genes covered by more CpGs on the microarray. Finally, our analysis was performed using sperm samples collected decades after conception of the veterans' children; a temporal difference in data that can only be avoided with a prospective, longitudinal study. Nevertheless, our findings suggest that the effects of parental trauma may be transmitted to future generations.

Ethics approval and consent to participate

This study was approved by the Greenslopes Research and Ethics Committee, Department of Veterans Affairs Human Research Ethics Committee, and Queensland University of Technology (QUT) Human Research Ethics Committee (1400000316). All participants provided written informed consent.

Conflicts of interest

All authors report no potential conflicts of interest.

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Abbreviations

PTSD	Posttraumatic stress disorder
CpG	cytosine-phosphate-guanine dinucleotide
HPA	hypothalamic pituitary adrenal
NR3C1	glucocorticoid receptor gene
GR	glucocorticoid receptor
FKBP5	FK506 Binding Protein 5
GMRF	Gallipoli Medical Research Foundation
QUT	Queensland University of Technology
GPH	Greenslopes Private Hospital
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

CAPS-5	Clinician Administered PTSD Scale for DSM-5
MINI	Mini International Neuropsychiatric Interview DSM IV
DASS-21	Depression Anxiety Stress Scale 21
PHQ-9	Patient Health Questionnaire PHQ-9
PRIME-MD	Primary Care Evaluation of Mental Disorders
MDD	Major Depressive Disorder
CD RISC	Connor-Davidson Resilience Scale
HCl	Hydrogen Chloride
EDTA	Ethylenediaminetetraacetic acid
NaCl	Sodium Chloride
SDS	Sodium Dodecyl Sulfate
DTT	Dithiothreitol
AGRF	Australian Genome Research Facility AGRF
SE	standard error
KEGG	Kyoto Encyclopedia of Genes and Genomes
VWDE	von Willebrand factor D and EGF domains
IHBI	Institute of Health and Biomedical Innovation

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.06.001.

References

Ara, H., Takagishi, M., Enomoto, A., Asai, M., Ushida, K., Asai, N., et al., 2016. Role for Daple in non-canonical Wnt signaling during gastric cancer invasion and metastasis. Cancer Sci. 107 (2), 133–139.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., et al., 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30 (10), 1363–1369.

Barfield, R.T., Almli, L.M., Kilaru, V., Smith, A.K., Mercer, K.B., Duncan, R., et al., 2014. Accounting for population stratification in DNA methylation studies. Genet. Epidemiol. 38 (3), 231–241.

Bezdjian, S., Schneider, K.G., Burchett, D., Baker, M.T., Garb, H.N., 2016. Resilience in the United States air force: psychometric properties of the connor-Davidson resilience scale (CD-RISC). Psychol. Assess. 29 (5), 479–485.

Breslau, N., 1999. Are baseline depressive symptoms associated with smoking initiation in adolescents? West. J. Med. 170 (5), 265.

Breslau, N., Davis, G.C., 1992. Posttraumatic stress disorder in an urban population of young adults: risk factors for chronicity. Am. J. Psychiatry 149 (5), 671–675.

Breslau, N., Kessler, R.C., 2001. The stressor criterion in DSM-IV posttraumatic stress disorder: an empirical investigation. Biol. Psychiatry 50 (9), 699–704.

Brewin, C.R., Andrews, B., Valentine, J.D., 2000. Meta-analysis of risk factors for post-traumatic stress disorder in trauma-exposed adults. J. Consult. Clin. Psychol. 68 (5), 748–766.

Bruenig, D., Mehta, D., Morris, C.P., Harvey, W., Lawford, B., Young, R.M., et al., 2017a. Genetic and serum biomarker evidence for a relationship between TNFalpha and PTSD in Vietnam war combat veterans. Compr. Psychiatr. 74, 125–133.

Bruenig, D., Morris, C.P., Mehta, D., Harvey, W., Lawford, B., Young, R.M., et al., 2017b. Nitric oxide pathway genes (NOS1AP and NOS1) are involved in PTSD severity, depression, anxiety, stress and resilience. Gene 625, 42–48.

Camprubi, C., Cigliano, R.A., Salas-Huetos, A., Garrido, N., Blanco, J., 2017. What the human sperm methylome tells us. Epigenomics 9 (10), 1299–1315.

Catoire, H., Sarayloo, F., Mourabit Amari, K., Apuzzo, S., Grant, A., Rochefort, D., et al., 2018. A direct interaction between two Restless Legs Syndrome predisposing genes: MEIS1 and SKOR1. Sci. Rep. 8 (1), 12173.

Connor, K.M., Davidson, J.R.T., 2003. Development of a new resilience scale: the connor-Davidson resilience scale (CD-RISC). Depress. Anxiety 18 (2), 76–82.

de Chevigny, A., Core, N., Follert, P., Wild, S., Bosio, A., Yoshikawa, K., et al., 2012. Dynamic expression of the pro-dopaminergic transcription factors Pax6 and Dlx2 during postnatal olfactory bulb neurogenesis. Front. Cell. Neurosci. 6, 6.

Dias, B.G., Ressler, K.J., 2014. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nat. Neurosci. 17 (1), 89–96.

Drielsma, A., Jalas, C., Simonis, N., Desir, J., Simanovsky, N., Pirson, I., et al., 2012. Two novel CCDC88C mutations confirm the role of DAPLE in autosomal recessive congenital hydrocephalus. J. Med. Genet. 49 (11), 708–712.

Franklin, T.B., Russig, H., Weiss, I.C., Graff, J., Linder, N., Michalon, A., et al., 2010. Epigenetic transmission of the impact of early stress across generations. Biol. Psychiatry 68 (5), 408–415.

Gapp, K., Jawaid, A., Sarkies, P., Bohacek, J., Pelczar, P., Prados, J., et al., 2014. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat. Neurosci. 17 (5), 667–669.

Gosenca, D., Kellert, B., Metzgeroth, G., Haferlach, C., Fabarius, A., Schwaab, J., et al., 2014. Identification and functional characterization of imatinib-sensitive DTD1-PDGFRB and CCDC88C-PDGFRB fusion genes in eosinophilia-associated myeloid/lymphoid neoplasms. Genes Chromosomes Cancer 53 (5), 411–421.

Hayashi, K., Kubo, K., Kitazawa, A., Nakajima, K., 2015. Cellular dynamics of neuronal migration in the hippocampus. Front. Neurosci. 9, 135.

Jawinski, P., Kirsten, H., Sander, C., Spada, J., Ulke, C., Huang, J., et al., 2018. Human brain arousal in the resting state: a genome-wide association study. Mol. Psychiatry.

Kaufman, J., Wymbs, N.F., Montalvo-Ortiz, J.L., Orr, C., Albaugh, M.D., Althoff, R., et al., 2018. Methylation in OTX2 and related genes, maltreatment, and depression in children. Neuropsychopharmacology 43 (11), 2204–2211.

Khulan, B., Manning, J.R., Dunbar, D.R., Seckl, J.R., Raikkonen, K., Eriksson, J.G., et al., 2014. Epigenomic profiling of men exposed to early-life stress reveals DNA methylation differences in association with current mental state. Transl. Psychiatry 4, e448.

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J.C., Pariante, C.M., et al., 2013. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat. Neurosci. 16 (1), 33–41.

Klengel, T., Pape, J., Binder, E.B., Mehta, D., 2014. The role of DNA methylation in stress-related psychiatric disorders. Neuropharmacology 80, 115–132.

Konjevod, M., Tudor, L., Svob Strac, D., Nedic Erjavec, G., Barbas, C., Zarkovic, N., et al., 2019. Metabolomic and glycomic findings in posttraumatic stress disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 88, 181–193.

Kuan, P.F., Waszczuk, M.A., Kotov, R., Clouston, S., Yang, X., Singh, P.K., et al., 2017. Gene expression associated with PTSD in World Trade Center responders: an RNA sequencing study. Transl. Psychiatry 7 (12), 1297.

Lecrubier, Y., Sheehan, D.V., Weiller, E., Amorim, P., Bonora, I., Harnett Sheehan, K., et al., 1997. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. Eur. Psychiatry 12 (5), 224–231.

Leek, J.T., Storey, J.D., 2007. Capturing heterogeneity in gene expression studies by surrogate variable analysis. PLoS Genet. 3 (9), 1724–1735.

Li, N., Zhao, C.T., Wang, Y., Yuan, X.B., 2010. The transcription factor Cux1 regulates dendritic morphology of cortical pyramidal neurons. PLoS One 5 (5) e10596.

Li, S., Papale, L.A., Zhang, Q., Madrid, A., Chen, L., Chopra, P., et al., 2016. Genome-wide alterations in hippocampal 5-hydroxymethylcytosine links plasticity genes to acute stress. Neurobiol. Dis. 86, 99–108.

Li, Y., Li, M., Liu, Y., Song, G., Liu, N., 2012. A microarray for microRNA profiling in spermatozoa from adult men living in an environmentally polluted site. Bull. Environ. Contam. Toxicol. 89 (6), 1111–1114.

Lovibond, S.H., Lovibond, P.F., 1995. The structure of negative emotional states: comparison of the depression anxiety stress scales (DASS) with the beck depression and anxiety inventories. Behav. Res. Ther. 33 (3), 335–343.

Luo, S.X., Timbang, L., Kim, J.I., Shang, Y., Sandoval, K., Tang, A.A., et al., 2016. TGF-beta signaling in dopaminergic neurons regulates dendritic growth, excitatory-inhibitory synaptic balance, and reversal learning. Cell Rep. 17 (12), 3233–3245.

Marczylo, E.L., Amoako, A.A., Konje, J.C., Gant, T.W., Marczylo, T.H., 2012. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? Epigenetics 7 (5), 432–439.

Maystadt, I., Rezsohazy, R., Barkats, M., Duque, S., Vannuffel, P., Remacle, S., et al., 2007. The nuclear factor kappaB-activator gene PLEKHG5 is mutated in a form of autosomal recessive lower motor neuron disease with childhood onset. Am. J. Hum. Genet. 81 (1), 67–76.

McLeay, S.C., Harvey, W.M., Romaniuk, M.N., Crawford, D.H., Colquhoun, D.M., Young, R.M., et al., 2017. Physical comorbidities of post-traumatic stress disorder in Australian Vietnam War veterans. Med. J. Aust. 206 (6), 251–257.

Mehta, D., Bruenig, D., Carrillo-Roa, T., Lawford, B., Harvey, W., Morris, C.P., et al., 2017. Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. Acta Psychiatr. Scand. 136 (5), 493–505.

Mehta, D., Gonik, M., Klengel, T., Rex-Haffner, M., Menke, A., Rubel, J., et al., 2011. Using polymorphisms in FKBP5 to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies. Arch. Gen. Psychiatr. 68 (9), 901–910.

Mehta, D., Klengel, T., Conneely, K.N., Smith, A.K., Altmann, A., Pace, T.W., et al., 2013. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. Proc. Natl. Acad. Sci. U. S. A. 110 (20), 8302–8307.

Mehta, D., Tropf, F.C., Gratten, J., Bakshi, A., Zhu, Z., Bacanu, S.A., et al., 2016. Evidence for genetic overlap between schizophrenia and age at first birth in women. JAMA Psychiatry 73 (5), 497–505.

Mehta, D., Voisey, J., Bruenig, D., Harvey, W., Morris, P.C., Lawford, B., et al., 2018. Transcriptome analysis reveals novel genes and immune networks dysregulated in veterans with PTSD. Brain Behav. Immun. 74, 133–142.

Morgan, C.P., Bale, T.L., 2011. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. J. Neurosci. 31 (33), 11748–11755.

O'Toole, B.I., Burton, M.J., Rothwell, A., Outram, S., Dadds, M., Catts, S.V., 2017. Intergenerational transmission of post-traumatic stress disorder in Australian Vietnam veterans' families. Acta Psychiatr. Scand. 135 (5), 363–372.

Perroud, N., Rutembesa, E., Paoloni-Giacobino, A., Mutabaruka, J., Mutesa, L., Stenz, L., et al., 2014. The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis. World J. Biol. Psychiatr. 15 (4), 334–345.

Robicsek, O., Makhoul, B., Klein, E., Brenner, B., Sarig, G., 2011. Hypercoagulation in chronic post-traumatic stress disorder. Isr. Med. Assoc. J. 13 (9), 548–552.

Rodgers, A.B., Bale, T.L., 2015. Germ cell origins of posttraumatic stress disorder risk: the transgenerational impact of parental stress experience. Biol. Psychiatry 78 (5), 307–314.

Ruggeri, G., Timms, A.E., Cheng, C., Weiss, A., Kollros, P., Chapman, T., et al., 2018. Biallelic mutations of CCDC88C are a rare cause of severe congenital hydrocephalus. Am. J. Med. Genet. 176 (3), 676–681.

Sasayama, D., Hiraishi, A., Tatsumi, M., Kamijima, K., Ikeda, M., Umene-Nakano, W.,

et al., 2013. Possible association of CUX1 gene polymorphisms with antidepressant response in major depressive disorder. Pharmacogenomics J. 13 (4), 354–358.
Sheehan, D.V., Lecrubier, Y., Harnett Sheehan, K., Janavs, J., Weiller, E., Keskiner, A., et al., 1997. The validity of the Mini international neuropsychiatric interview (MINI) according to the SCID-P and its reliability. Eur. Psychiatry 12 (5), 232–241.
Spitzer, R.L., Kroenke, K., Williams, J.B., 1999. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care evaluation of mental disorders. Patient health questionnaire. J. Am. Med. Assoc. 282 (18), 1737–1744.
Tsoi, H., Yu, A.C., Chen, Z.S., Ng, N.K., Chan, A.Y., Yuen, L.Y., et al., 2014. A novel missense mutation in CCDC88C activates the JNK pathway and causes a dominant form of spinocerebellar ataxia. J. Med. Genet. 51 (9), 590–595.
Wabnitz, G.H., Michalke, F., Stober, C., Kirchgessner, H., Jahraus, B., van den Boomen, D.J., et al., 2011. L-plastin phosphorylation: a novel target for the immunosuppressive drug dexamethasone in primary human T cells. Eur. J. Immunol. 41 (11), 3157–3169.
Wang, J., Duncan, D., Shi, Z., Zhang, B., 2013. WEB-based GEne SeT AnaLysis toolkit (WebGestalt): update 2013. Nucleic Acids Res. 41, W77–W83 Web Server issue.
Weathers, F.W., Marx, B.P., Friedman, M.J., Schnurr, P.P., 2014. Posttraumatic stress disorder in DSM-5: new criteria, new measures, and implications for assessment. Psychological Injury and Law 7 (2), 93–107.
Wehmer, F., Porter, R.H., Scales, B., 1970. Pre-mating and pregnancy stress in rats affects behaviour of grandpups. Nature 227 (5258), 622.
Wockner, L.F., Noble, E.P., Lawford, B.R., Young, R.M., Morris, C.P., Whitehall, V.L., et al., 2014. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. Transl. Psychiatry 4, e339.
Yang, B.Z., Zhang, H., Ge, W., Weder, N., Douglas-Palumberi, H., Perepletchikova, F., et al., 2013. Child abuse and epigenetic mechanisms of disease risk. Am. J. Prev. Med. 44 (2), 101–107.
Yehuda, R., 2004. Risk and resilience in posttraumatic stress disorder. J. Clin. Psychiatry 65 (Suppl. 1), 29–36.
Yehuda, R., Daskalakis, N.P., Bierer, L.M., Bader, H.N., Klengel, T., Holsboer, F., et al., 2016. Holocaust exposure induced intergenerational effects on FKBP5 methylation. Biol. Psychiatry 80 (5), 372–380.
Yehuda, R., Daskalakis, N.P., Lehrner, A., Desarnaud, F., Bader, H.N., Makotkine, I., et al., 2014a. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. Am. J. Psychiatry 171 (8), 872–880.
Yehuda, R., Lehrner, A., 2018. Intergenerational transmission of trauma effects: putative role of epigenetic mechanisms. World Psychiatr. 17 (3), 243–257.
Yehuda, R., Pratchett, L.C., Elmes, M.W., Lehrner, A., Daskalakis, N.P., Koch, E., et al., 2014b. Glucocorticoid-related predictors and correlates of post-traumatic stress disorder treatment response in combat veterans. Interface Focus 4 (5), 20140048.
Yehuda, R., Teicher, M.H., Seckl, J.R., Grossman, R.A., Morris, A., Bierer, L.M., 2007. Parental posttraumatic stress disorder as a vulnerability factor for low cortisol trait in offspring of holocaust survivors. Arch. Gen. Psychiatr. 64 (9), 1040–1048.