HHS Public Access

Author manuscript

Psychiatry Clin Neurosci. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

Psychiatry Clin Neurosci. 2019 June ; 73(6): 323–330. doi:10.1111/pcn.12835.

Genome-wide DNA methylation investigation of glucocorticoid exposure within buccal samples

Patricia R. Braun, Ph.D.[a,d], **Mai Tanaka-Sahker, D.D.S.**[a], **Aubrey C. Chan, M.D., Ph.D.**[a], **Sydney S. Jellison, H.S.D.G.**[a], **Mason J. Klisares, H.S.D.G.**[a], **Benjamin W. Hing, Ph.D.**[a], **Yaseen Shabbir, H.S.D.G.**[a], **Lindsey N. Gaul, B.A.**[a], **Yasunori Nagahama, M.D.**[b], **Julian Robles, B.A.**[a], **Jonathan T. Heinzman, B.A.**[a], **Sayeh Sabbagh, M.S.W.**[a], **Ellyn M. Cramer, H.S.D.G.**[a], **Gabrielle N. Duncan, H.S.D.G.**[a], **Kumi Yuki, M.D.**[a], **Liesl N. Close, M.D.**[b], **Brian J. Dlouhy, M.D.**[b], **Matthew A. Howard III, M.D.**[b], **Hiroto Kawasaki, M.D.**[b], **Kyle M. Stein, M.D.**[c], **James B. Potash, M.D., M.P.H.**[d], and **Gen Shinozaki, M.D.**[a,b,e,f]

[a]Department of Psychiatry, University of Iowa Carver College of Medicine, 200 Hawkins Dr, Iowa City, IA 52246, USA

[b]Department of Neurosurgery, University of Iowa Carver College of Medicine, 200 Hawkins Dr, Iowa City, IA 52246, USA

[c]Department of Oral and Maxillofacial Surgery, University of Iowa Carver College of Medicine, 200 Hawkins Dr, Iowa City, IA 55246, USA

[d]Department of Psychiatry, Johns Hopkins University School of Medicine, 1800 Orleans St, Baltimore, MD 21287, USA

[e]Iowa Neuroscience Institute, University of Iowa Carver College of Medicine, Iowa City, IA 52246, USA

[f]Interdisciplinary Graduate Program for Neuroscience, University of Iowa Carver College of Medicine, Iowa City, IA 52246, USA

Abstract

Aim: Glucocorticoids play a major role in regulating the stress response, and an imbalance of glucocorticoids has been implicated in stress-related disorders. Within mouse models, CpGs across the genome have been shown to be differentially methylated in response to glucocorticoid treatment, and using the Infinium 27K array, it was shown that humans given synthetic

Corresponding Author: Gen Shinozaki, gen-shinozaki@uiowa.edu, Address: Department of Psychiatry, University of Iowa Hospitals and Clinics, 200 Hawkins Dr, Iowa City, IA, 52246, Phone number: 319-384-4932.

Author Contributions

GS planned the study, assembled the study team, and oversaw the entire study process. GS, JBP, BWH, ACC, KMS, and PRB designed the study. MTS and SS headed the subject enrollment. KY, MJK, SSJ, LNG, JR, JTH, EMC, GND, and YS acquired and processed the samples from the dental cohort. YN, LNC, BJD, MAH, and HK performed the neurosurgical resections and provided tissue samples. PRB performed the methylome assays and analyzed the data. PRB, GS, and JBP wrote the manuscript. All authors read and approved the final manuscript.

Within the *Psychiatry and Clinical Neurosciences* journal, this manuscript belongs in the field of molecular psychiatry and psychobiology.

Disclosure Statement

GS is a founder of Predelix Medical LLC, although that entity has no relevance or relationship to this manuscript. All other authors declare no conflicts of interest.

glucocorticoids had DNA methylation (DNAm) changes in blood. However, further investigation of the extent to which glucocorticoids affect DNAm across a larger proportion of the genome is needed.

Methods: Buccal samples were collected before and after synthetic glucocorticoid treatment in the context of dental procedure. This included 30 tooth extraction surgery patients who received 10 mg of dexamethasone. Genome-wide DNAm was assessed with the Infinium HumanMethylationEPIC array.

Results: Five CpGs showed genome-wide significant DNAm changes that was >10%. These differentially methylated CpGs were in or nearest the following genes: *ZNF438*, *KLHDC10*, miR-544 or *CRABP1*, *DPH5*, and *WDFY2*. Using previously published datasets of human blood gene expression changes following dexamethasone exposure, a significant proportion of genes with FDR-adjusted significant CpGs were also differentially expressed. A pathway analysis of the genes with FDR-adjusted significant CpGs revealed significant enrichment of olfactory transduction, pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and steroid hormone biosynthesis pathways.

Conclusion: High-dose synthetic glucocorticoid administration in the setting of dental procedure was significantly associated with DNAm changes within buccal samples. These findings are consistent with prior findings of an influence of glucocorticoids on DNAm in humans.

Keywords

DNA methylation; Epigenomics; Glucocorticoids; Stress (Psychological); Dexamethasone

Introduction

Stress is a major contributing factor in the development of psychiatric disorders [1, 2]. Major depressive disorder (MDD) has been linked to stress [1, 3], with the incidence of a depressive episode increased with a major stressful event [4]. Approximately 17% of the population is affected by MDD and about 30% of patients do not undergo remission and suffer from chronic depression despite treatment [5, 6]. Post-traumatic stress disorder (PTSD) is precipitated by a traumatic event, and it affects 5–10% of the population [7].

The hypothalamic-pituitary-adrenal (HPA) axis plays an essential role in mediating the stress response, which ultimately results in the release of glucocorticoids (GCs) from the adrenal cortex that regulate tissues throughout the body through the binding of GCs to the glucocorticoid receptor (GR) [8]. Dysregulation of this system is often seen with chronic stress and in psychiatric disorders [9, 10]. PTSD has been associated with suppressed cortisol levels, which can lead to hypersensitivity of the GR and enhanced negative feedback inhibition [11]. In contrast, in MDD, blunted cortisol and adrenocorticotropic hormone responses are often present [12]. These alterations in GC feedback on the HPA axis constitute one of the most consistent biomarkers for MDD and PTSD [13]. The contribution of GCs to psychiatric disorders is further evidenced in a large epidemiological study of individuals given GC treatment for a range of inflammatory disorders [14]. Individuals treated with GCs had an elevated risk of suicide or suicide attempt, depression, mania, delirium, and panic

disorder [14]. This is consistent with the hypothesis that the impact of stress on psychiatric disorders is mediated in part by GC exposure.

MDD, PTSD, and stressful life events have all been associated with changes in DNA methylation (DNAm) [15–17]. To tease out the role of GCs themselves, rodent models of GC exposure have been employed. One study reported DNAm changes in *Fkbp5*, the co-chaperone of the GR, within the hippocampus [18], which was later found to correlate partially with similar changes in blood [19]. A genome-wide investigation of GC exposure in mice found differentially methylated regions (DMRs) across the genome, and 209 DMRs that mapped to the same genomic coordinates in the hippocampus and blood were significantly changed with GC exposure in the same direction within both tissues [20].

Only one study, to our knowledge, has previously investigated genome-wide DNAm changes in response to synthetic glucocorticoid administration in humans. In that study, investigators examined patients with chronic obstructive pulmonary disease (COPD) and made a DNAm comparison using blood samples from those who were given glucocorticoid treatment and those who were not. The study was performed with the Illumina 27K methylation array [21]. A total of 511 sites were significantly differentially methylated, and an enrichment of corresponding genes involved in membrane components, hemostasis and coagulation, cellular ion homeostasis, leukocyte and lymphocyte activation, and protein transport was found [21].

To better understand the impact of GC exposure on genome-wide methylation within humans, we investigated DNAm changes by comparing buccal samples taken before and after individuals underwent tooth extraction and were given a synthetic GC, dexamethasone, prior to the procedure. We employed the Illumina EPIC array, which assays >850,000 CpG sites, and we thus substantially expanded the coverage of the genomic landscape compared to earlier work.

Methods

Participants and sample collection

Two cohorts of individuals exposed to synthetic glucocorticoids were used and both were approved by the University of Iowa's Human Subjects Research Institutional Review Board. Written informed consent was obtained. The first cohort included individuals recruited from the dental clinic (DC). These subjects underwent tooth extraction procedure, and 10 mg of dexamethasone was administered prior to surgery. From 30 individuals, buccal tissue was collected with swabs (Puritan, 25–1506 1PF TT MC) before and after dental surgery. Buccal samples were chosen because they are more easily obtained than blood samples, and the dental procedure patients had difficulty producing saliva for sampling. Subject characteristics from the DC cohort are in Table 1. Depressive symptoms and childhood adversity were measured with the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR-16) scale and the Adverse Childhood Experiences (ACE) questionnaires, respectively.

The second cohort (NSG) included twenty-one subjects with medically intractable epilepsy undergoing neurosurgery who were recruited for a separate study between March 2014 and April 2017 at the University of Iowa Hospitals and Clinics. Before and after dexamethasone exposure, whole blood samples were collected in EDTA tubes, saliva samples with the Oragene DISCOVER™ kit (DNA Genotek Inc., OGR-500), and buccal tissue with swabs (Puritan, 25–1506 1PF TT MC). Resected brain tissue samples were taken within the operating room before dexamethasone exposure and between 30 minutes to 3 hours after. In total, both pre- and post-samples were collected for blood (n = 18), buccal tissue (n = 13), saliva (n = 21), and brain (n = 10). Samples were immediately stored and transported on dry ice and a portion of each brain region was sent to pathology to exclude tissues with malignancy. All samples were stored at −80°C. NSG sample characteristics are in Table 2.

Methylome assays

Genomic DNA was extracted from whole blood, buccal, saliva, and brain tissues with the MasterPure™ DNA extraction kit (Epicenter, MCD85201) following the respective protocols for each tissue type. DNA quality was assessed with NanoDrop spectrometry and quantified with the Qubit™ dsDNA Broad Range Assay Kit (ThermoFisher Scientific, Q32850). For each sample, 500 ng of DNA was bisulfite converted with the EZ DNA Methylation™ Kit (Zymo Research, D5002). The Infinium HumanMethylationEPIC BeadChip™ Kit (Illumina, WG-317–1002) was used to analyze genome-wide DNAm in all brain, blood, saliva, and buccal samples. The arrays were scanned with the Illumina iScan platform.

The methylation data were processed with the R packages Minfi and RnBeads [22–24]. The two cohorts were analyzed separately. Background correction was performed with the Noob method in Minfi [23, 25]. Using RnBeads, probes were filtered out if they: 1) overlapped within 5 bp of a SNP (DC: 21,414; NSG: 21,358 probes), 2) had a detection *P*-value > 0.01 or were considered unreliable measures based on RnBeads's greedy-cut algorithm (DC: 26,065; NSG: 18,864 probes), or 3) were context-specific sites (DC: 2,825; NSG: 2,873 probes). After filtering, 816,532 probes remained in the DC cohort and 822,996 in the NSG cohort. Samples were normalized with beta mixture quantile dilation (BMIQ) [26]. Samples from the same individual were verified by generating a heatmap cluster of the 65 SNP probes on the array.

Statistical analysis

All statistical analyses were performed in R [27]. Differential methylation was assessed with RnBeads using the limma method with a paired analysis by subject [24, 28]. A hierarchical linear model was fitted with the empirical Bayes approach. Covariates included age, sex, and surrogate variables estimated with the "be" method [24, 29]. Fisher's exact test was performed for the enrichment of glucocorticoid expressed genes. The R package missMethyl was used for pathway analysis with KEGG-defined pathways, and it employs a hypergeometric test taking into account the number of probes per gene [30]. For the comparison of DNAm from buccal and brain tissues, the correlation coefficient and associated p-value were calculated with Spearman's test.

Results

Genome-wide analysis of buccal samples from dental procedure patients

Thirty individuals were recruited from the dental clinic, and buccal samples were taken before and after tooth extraction procedure. Subject characteristics can be found in Table 1. Average age was 25.6 ± 7.6 years old. The QIDS-SR depressive symptom score average was 7.6 ± 4.9, and the ACE average score was 2.3 ± 2.3, indicating a relatively young and healthy population. DNA samples were run on the Illumina EPIC array, and DNAm differences between pre- and post-dexamethasone samples were analyzed with the limma method in RnBeads, with a paired analysis adjusted for age, sex, and estimated surrogate variables.

For the analysis of individual CpGs, we set a Bonferroni genome-wide significance threshold for this experiment at $p < 6.12 \times 10^{-8}$ based on the 816,532 probes left after preprocessing, and we further only considered CpG changes meaningful if they showed DNAm differences greater than 10%. Figure 1 shows the distribution of individual CpG differences and their corresponding raw p-value. Five CpGs were genome-wide significant with differences greater than 10%, and all five also showed a decrease in methylation after dexamethasone exposure (Table 3). The top finding is an intergenic CpG closest to the zinc finger protein 438 (*ZNF438*; average DNAm: pre-steroid 68%, post-steroid 54%; p-value = 3.21×10^{-9}). Subsequent top findings that surpassed the Bonferroni threshold included CpGs in or near the following genes: *KLHDC10* (average DNAm: pre-steroid 85%, post-steroid 74%; p-value = 1.11×10^{-8}), miR-544 or *CRABP1* (average DNAm: pre-steroid 82%, post-steroid 70%; p-value = 1.56×10^{-8}), *DPH5* (average DNAm: pre-steroid 73%, post-steroid 58%; p-value = 3.66×10^{-8}), and *WDFY2* (average DNAm: pre-steroid 92%, post-steroid 81%; p-value = 4.36×10^{-8}). For a more comprehensive list of the top differential methylation results, Supplemental Table 1 contains all the CpGs with the less conservative FDR-adjusted p-value of 0.01 and differences greater than 10%.

We further investigated whether psychiatric risk genes showed evidence for differential methylation from dexamethasone administration. In this analysis, CpGs were considered significant if their FDR-adjusted p-value was < 0.05 and they had differences greater than 10%. Among the genes considered (FK506 binding protein 5 [*FKBP5*], the glucocorticoid receptor [*NR3C1*], brain-derived neurotrophic factor [*BDNF*], the serotonin transporter [*SLC6A4*], and corticotropin-releasing hormone [*CRH*]), there were three significant CpGs in *FKBP5*, five in *NR3C1*, and one in *BDNF* (Table 4).

Additionally, the genes implicated in GWASs of schizophrenia, bipolar disorder, and MDD (N=366) were analyzed (Supplemental Table 2)[31–33]. 108 of the genes had at least one CpG that was differentially methylated, and 28 genes had at least one CpG that was differentially methylated and showed correlation between buccal and brain tissue DNAm. This included 240 individual CpGs that were differentially methylated, and 35 that were differentially methylated and correlated (Supplemental Table 3).

Pathway analysis

A pathway analysis was performed with KEGG-defined pathways using the missMethyl R package to correct for the different numbers of probes per gene on the array [30]. Input for the analysis included CpGs with differences greater than 10% and FDR-adjusted p-values < 0.01 (N = 2,587). Top pathways included olfactory transduction (FDR-adjusted p-value = 1.15×10^{-3}), pentose and glucuronate interconversions (FDR-adjusted p-value = 4.31×10^{-3}), ascorbate and aldarate metabolism (FDR-adjusted p-value = 4.31×10^{-3}), steroid hormone biosynthesis (FDR-adjusted p-value = 4.31×10^{-3}), and retinol metabolism (FDR-adjusted p-value = 0.11; Table 5).

Overlap with genes differentially expressed following GC exposure

Differential gene expression within blood samples following administration of 1.5 mg dexamethasone has been investigated in two human studies [34, 35]. These studies yielded 1,052 genes that were differentially expressed in response to dexamethasone across both studies and that are covered on the Illumina EPIC array. As with the pathway analysis, genes that were included had at least one CpG that was differentially methylated in our data at FDR-adjusted p-value < 0.01 and a DNAm difference > 10%. Of these, 154 genes (15%) also showed a significant proportion with differential methylation after dexamethasone exposure within our study (Fisher's exact p-value = 8.63×10^{-4}; Supplemental Table 4).

Analysis of dexamethasone exposure in neurosurgery cohort

We performed an additional study in a separate cohort of patients given dexamethasone. In this cohort, patients undergoing neurosurgical brain resection for treatment refractory epilepsy were administered dexamethasone during surgery. Blood, buccal, saliva, and brain samples were taken before and after dexamethasone exposure. No genome-wide significant changes in DNAm were found from any of the tissues, though the top differentially methylated CpGs are listed in Supplemental Table 5. Of the CpGs with FDR-adjusted p-value < 0.01 and differences > 10% from the dental procedure cohort, a subset replicated in additional tissues with differences greater than 10% and at nominal significance. This included 13 CpGs identified in samples from blood, 5 from buccal tissue, 58 from saliva, and 6 from brain (Supplemental Table 6). One CpG in the gene regulating synaptic membrane exocytosis 2 (*RIMS2*) was significant in all three peripheral tissues.

Correlation of buccal DNA methylation of top findings to brain tissue DNA methylation

With the NSG cohort in this paper, a separate analysis was performed of the five genome-side significant results, assessing the degree of correlation between DNAm of buccal and brain tissues (n = 21), using a web-based resource we created, IMAGE-CpG (https://han-lab.org/methylation/default/imageCpG) [36]. We observed non-significant moderate correlation between brain and buccal DNAm for the CpG in *WDFY2* (cg06335209; rho = 0.5, p-value = 0.07), and low-moderate correlation for the CpG in *KLHDC10* (cg01119284; rho = 0.3, p-value = 0.27).

Discussion

This study assessed the impact of synthetic glucocorticoids on DNAm within humans. Studies in mice have demonstrated that chronic glucocorticoid exposure evokes DNAm changes across the genome in hippocampal and blood tissues [20]. In a DNAm comparison of COPD patients with and without glucocorticoid treatment DNAm differences across 511 CpGs were found in blood samples using the Illumina 27K array [21]. Individuals with Cushing's syndrome have sustained elevated glucocorticoid levels, and in a case-control Illumina 450K array study, additional DNAm differences were found to be associated with this disorder [37]. Our study builds upon these reports by demonstrating across a larger portion of the genome DNAm changes that occur within individuals exposed to a single high dose of glucocorticoids.

Among the top differentially methylated CpGs in this study was one whose nearest gene is *ZNF438,* a gene implicated in transcriptional inhibition and in regulation of the immune system [38, 39], and both transcriptional control and the immune system are regulated by glucocorticoids. A decrease in methylation was also significant in a CpG within the gene that encodes the cellular retinoic acid binding protein 1 (CRABP1), a protein involved in retinoic acid-mediated proliferation and differentiation [40]. CRABP1 binds to and catalyzes the destruction of retinoic acid [41]. Of interest, in rats exposed to chronic unpredictable mild stress, *Crabp1* was decreased in the hypothalamus [42]. Though not FDR-significant, retinol metabolism was also one of the top pathways enriched from the dexamethasone-induced DNAm findings in this study. Retinoic acid regulates gene transcription and is important in processes including the development of the central nervous system [43] and neuronal plasticity [44–46]. Hyperactivity of the HPA axis along with anxious and depressive behaviors have been seen in rats treated with retinoic acid [47].

Intriguingly, steroid hormone biosynthesis was also among the top pathways, indicating that high dose glucocorticoid treatment may influence epigenetic regulation of the HPA axis. This is also supported by the differential methylation seen in *FKBP5* and *NR3C1*. In addition, steroid hormone biosynthesis involves the production of other cholesterol-derived hormones in addition to cortisol, including estradiol, aldosterone, and testosterone, all of which interact with and mediate the stress response [48–54]. The enrichment of steroid hormone biosynthesis genes suggests these genes may be regulated by dexamethasone-induced DNAm changes.

A major limitation of this study is the potential confounding of cell-specific DNAm effects. Buccal samples were used, and although they are primarily epithelial cells, contamination from saliva, which predominantly includes white blood cells, could impact methylation levels. Additionally, the sample size was limited (n = 30), and a replication of the findings within the same dental procedure context was not possible. Therefore, we included the additional NSG analysis that involves the analysis of three peripheral tissues and resected brain tissue. However, this cohort was also limited in sample size (blood (n = 18), buccal (n = 13), saliva (n = 21), and brain samples (n = 10), and differences with the DC cohort include different dexamethasone doses and timing of the post-sample collection. Furthermore, the brain regions resected were not uniform among all the subjects. Future

studies will be needed to determine the functional impact of the differential methylation findings in response to GC exposure.

Our study investigated DNAm differences across the genome with the Illumina EPIC array in individuals given synthetic glucocorticoids. Significant DNAm changes were observed in genes involved in transcriptional inhibition, the immune system, cellular proliferation, and retinoic acid metabolism. High doses of synthetic glucocorticoid exposure may also impact the HPA axis through epigenetic modifications in steroid hormone biosynthesis genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Support for GS was through a National Institutes of Health Research Career Development K Award K23MH107654. PRB received training funding from the National Institutes of Health Predoctoral Training Grant T32GM008629, PI Daniel Eberl.

References

1. Kessler R, Davis C, Kendler KS. Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. Psychological Medicine 1997;27(5):1101–9. [PubMed: 9300515]
2. Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. Am J Psychiatry 1999;156(6):837–41. [PubMed: 10360120]
3. Hammen C Stress and depression. Annu Rev Clin Psychol 2005;1:293–319. [PubMed: 17716090]
4. Paykel ES, Myers JK, Dienelt MN, Klerman GL, Lindenth JJ, Pepper MP. Life Events and Depression - a Controlled Study. Arch Gen Psychiat 1969;21(6):753. [PubMed: 5389659]
5. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 2003;289(23):3095–105. [PubMed: 12813115]
6. Fava M Diagnosis and definition of treatment-resistant depression. Biological Psychiatry 2003;53(8):649–59. [PubMed: 12706951]
7. Galea S, Nandi A, Vlahov D. The epidemiology of post-traumatic stress disorder after disasters. Epidemiol Rev 2005;27:78–91. [PubMed: 15958429]
8. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA 1992;267(9):1244–52. [PubMed: 1538563]
9. de Kloet CS, Vermetten E, Geuze E, Kavelaars A, Heijnen CJ, Westenberg HG. Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. J Psychiatr Res 2006;40(6):550–67. [PubMed: 16214171]
10. Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: insights from HPA axis studies in humans. Psychoneuroendocrinology 2008;33(6): 693–710. [PubMed: 18602762]
11. Yehuda R, Bierer LM, Andrew R, Schmeidler J, Seckl JR. Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors. J Psychiatr Res 2009;43(9):877–83. [PubMed: 19162277]
12. Dinan TG. Glucocorticoids and the genesis of depressive illness. A psychobiological mode. Br J Psychiatry 1994;164(3):365–71. [PubMed: 7832833]
13. Griffiths BB, Hunter RG. Neuroepigenetics of stress. Neuroscience 2014;275:420–35. [PubMed: 24976514]
14. Fardet L, Petersen I, Nazareth I. Suicidal behavior and severe neuropsychiatric disorders following glucocorticoid therapy in primary care. Am J Psychiatry 2012;169(5):491–7. [PubMed: 22764363]

15. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 2009;12(3):342–8. [PubMed: 19234457]
16. Davies MN, Krause L, Bell JT, Gao F, Ward KJ, Wu H, et al. Hypermethylation in the ZBTB20 gene is associated with major depressive disorder. Genome Biol 2014;15(4):R56. [PubMed: 24694013]
17. Maddox SA, Kilaru V, Shin J, Jovanovic T, Almli LM, Dias BG, et al. Estrogen-dependent association of HDAC4 with fear in female mice and women with PTSD. Mol Psychiatry 2017;00:1–8.
18. Lee RS, Tamashiro KL, Yang X, Purcell RH, Harvey A, Willour VL, et al. Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. Endocrinology 2010;151(9):4332–43. [PubMed: 20668026]
19. Ewald ER, Wand GS, Seifuddin F, Yang X, Tamashiro KL, Potash JB, et al. Alterations in DNA methylation of Fkbp5 as a determinant of blood-brain correlation of glucocorticoid exposure. Psychoneuroendocrinology 2014;44:112–22. [PubMed: 24767625]
20. Seifuddin F, Wand G, Cox O, Pirooznia M, Moody L, Yang X, et al. Genome-wide Methyl-Seq analysis of blood-brain targets of glucocorticoid exposure. Epigenetics 2017;8:637–52.
21. Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SI, et al. Systemic steroid exposure is associated with differential methylation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012;186(12):1248–55. [PubMed: 23065012]
22. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014;30(10):1369-.
23. Fortin J, Triche TJ Jr, Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. Bioinformatics 2017;33(4):558–60. [PubMed: 28035024]
24. Assenov Y, Muller F, Lutsik P, Walter J, Lengauer T, Bock C. Comprehensive analysis of DNA methylation data with RnBeads. Nat Methods 2014;11(11):1138–40. [PubMed: 25262207]
25. Triche TJ Jr, Weisenberger DJ, Van Den Berg D, Laird PW, Siegmund KD. Low-level processing of Illumina Infinium DNA methylation beadarrays. Nucleic Acids Research 2013;41(7):e90. [PubMed: 23476028]
26. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, et al. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics 2013;29(2):189–96. [PubMed: 23175756]
27. R Core Team. R: A language and environment for statistical computing 2017 [Available from: https://www.R-project.org.
28. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43(7):e47. [PubMed: 25605792]
29. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 2012;28(6):882–3. [PubMed: 22257669]
30. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. Bioinformatics 2016;32(2):286–8. [PubMed: 26424855]
31. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014;511(7510):421–7. [PubMed: 25056061]
32. Stahl E, Forstner AJ, McQuillin A, Ripke S, Trubetskoy V, Mattheisen M, et al. Genome-wide association study identifies 30 Loci associated with bipolar disorder. bioRxiv 2017.
33. Major Depressive Disorder Working Group, Wray NR, Sullivan PF. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depressive disorder. bioRxiv 2017.

34. Menke A, Arloth J, Gerber M, Rex-Haffner M, Uhr M, Holsboer F, et al. Dexamethasone stimulated gene expression in peripheral blood indicates glucocorticoid-receptor hypersensitivity in job-related exhaustion. Psychoneuroendocrinology 2014;44:35–46. [PubMed: 24767618]
35. Menke A, Arloth J, Putz B, Weber P, Klengel T, Mehta D, et al. Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients. Neuropsychopharmacology 2012;37(6):1455–64. [PubMed: 22237309]
36. Braun PR, Han S, Hing B, Nagahama Y, Gaul LN, Heinzman JT, et al. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. Transl Psychiatry 2019;9(1):47. [PubMed: 30705257]
37. Glad CA, Andersson-Assarsson JC, Berglund P, Bergthorsdottir R, Ragnarsson O, Johannsson G. Reduced DNA methylation and psychopathology following endogenous hypercortisolism - a genome-wide study. Sci Rep 2017;7:44445. [PubMed: 28300138]
38. Zhong Z, Wan B, Qiu Y, Ni J, Tang W, Chen X, et al. Identification of a novel human zinc finger gene, ZNF438, with transcription inhibition activity. J Biochem Mol Biol 2007;40(4):517–24. [PubMed: 17669267]
39. International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011;476(7359):214–9. [PubMed: 21833088]
40. Tang XH, Vivero M, Gudas LJ. Overexpression of CRABPI in suprabasal keratinocytes enhances the proliferation of epidermal basal keratinocytes in mouse skin topically treated with all-trans retinoic acid. Exp Cell Res 2008;314(1):38–51. [PubMed: 17727842]
41. Fiorella PD, Giguere V, Napoli JL. Expression of cellular retinoic acid-binding protein (Type II) in Escherichia coli: Characterization and comparison to cellular retinoic acid-binding protein (Type I). J Biol Chem 1993:21545–52. [PubMed: 8408005]
42. Chen XN, Meng QY, Bao AM, Swaab DF, Wang GH, Zhou JN. The involvement of retinoic acid receptor-alpha in corticotropin-releasing hormone gene expression and affective disorders. Biol Psychiatry 2009;66(9):832–9. [PubMed: 19596122]
43. Maden M, Holder N. The involvement of retinoic acid in the development of the vertebrate central nervous system. Dev Suppl 1991;Suppl:87–94. [PubMed: 1842359]
44. Aoto J, Nam CI, Poon MM, Ting P, Chen L. Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. Neuron 2008;60(2):308–20. [PubMed: 18957222]
45. Jacobs S, Lie DC, DeCicco KL, Shi Y, DeLuca LM, Gage FH, et al. Retinoic acid is required early during adult neurogenesis in the dentate gyrus. Proc Natl Acad Sci U S A 2006;103(10):3902–7. [PubMed: 16505366]
46. Wang HL, Zhang Z, Hintze M, Chen L. Decrease in calcium concentration triggers neuronal retinoic acid synthesis during homeostatic synaptic plasticity. J Neurosci 2011;31(49):17764–71. [PubMed: 22159093]
47. Cai L, Yan XB, Chen XN, Meng QY, Zhou JN. Chronic all-trans retinoic acid administration induced hyperactivity of HPA axis and behavioral changes in young rats. Eur Neuropsychopharmacol 2010;20(12):839–47. [PubMed: 20659790]
48. Kubzansky LD, Adler GK. Aldosterone: a forgotten mediator of the relationship between psychological stress and heart disease. Neurosci Biobehav Rev 2010;34(1):80–6. [PubMed: 19631234]
49. Hlavacova N, Jezova D. Chronic treatment with the mineralocorticoid hormone aldosterone results in increased anxiety-like behavior. Horm Behav 2008;54(1):90–7. [PubMed: 18377905]
50. Weiser MJ, Handa RJ. Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis via estrogen receptor alpha within the hypothalamus. Neuroscience 2009;159(2):883–95. [PubMed: 19166915]
51. Viau V, Meaney MJ. Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. Endocrinology 1991;129(5):2503–11. [PubMed: 1657578]
52. Lund TD, Munson DJ, Haldy ME, Handa RJ. Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. J Neuroendocrinol 2004;16(3):272–8. [PubMed: 15049858]

53. Handa RJ, Kudwa AE, Donner NC, McGivern RF, Brown R. Central 5-alpha reduction of testosterone is required for testosterone's inhibition of the hypothalamo-pituitary-adrenal axis response to restraint stress in adult male rats. Brain Res 2013;1529:74–82. [PubMed: 23880372]

54. Handa RJ, Nunley KM, Lorens SA, Louie JP, McGivern RF, Bollnow MR. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol Behav 1994;55(1):117–24. [PubMed: 8140154]

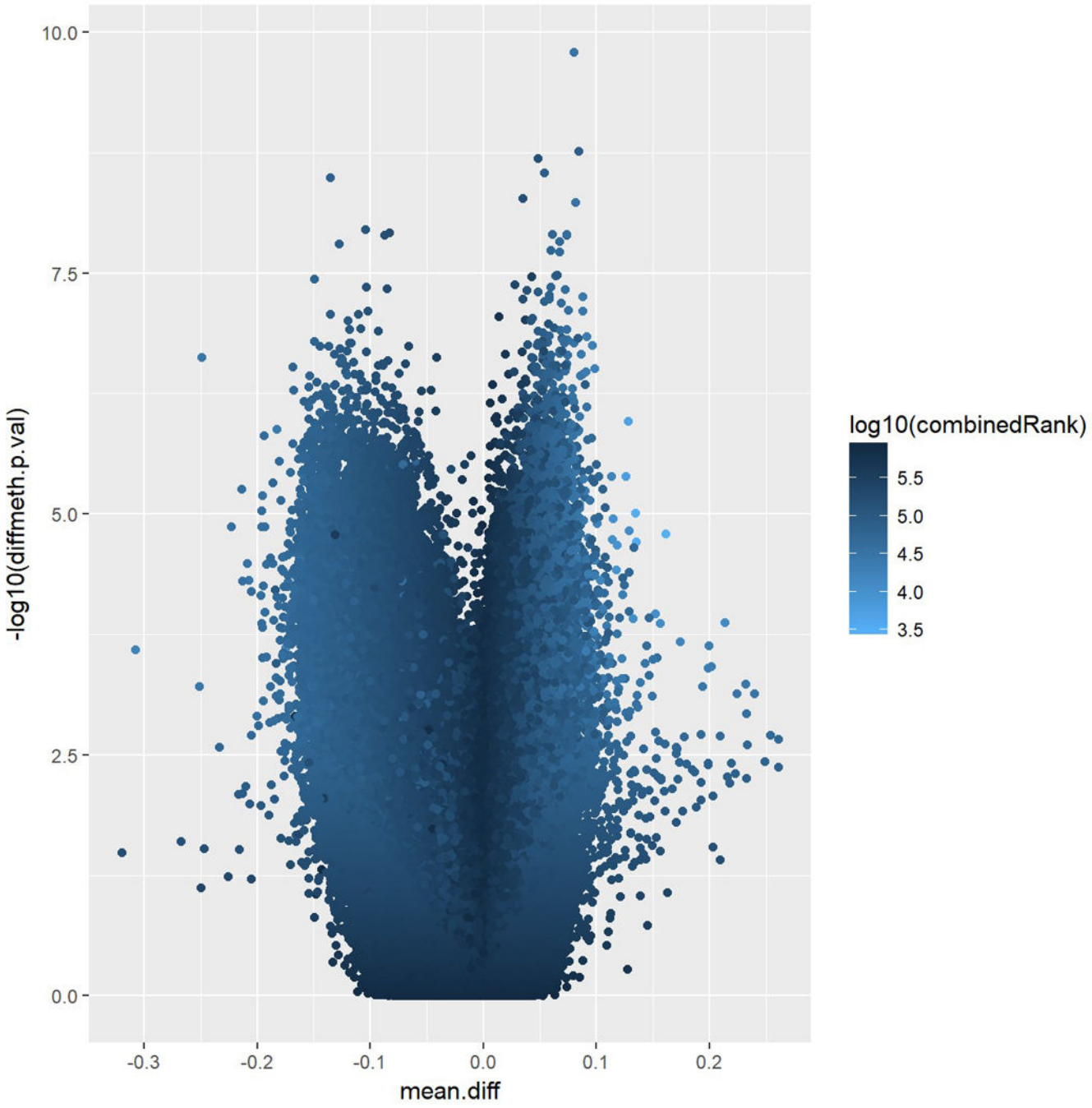

Figure 1.

The degree of significance of all individual CpGs represented on the Illumina EPIC array and their methylation differences after dexamethasone exposure in buccal samples from individuals who underwent tooth extraction (n = 30).

Table 1.

Clinical characteristics of the dental procedure study participants (n = 30)

Patient	Sex	Age	GC duration (min)	QIDS-SR_16	ACE
2	female	31	112	3	1
5	female	24	53	2	0
7	female	22	109	5	3
9	female	25	NA	NA	NA
13	male	26	118	5	1
14	female	19	106	4	0
16	male	22	118	15	5
17	female	21	170	15	3
18	female	36	134	NA	NA
19	female	25	115	9	10
20	male	28	NA	NA	NA
21	female	23	107	9	4
22	female	24	244	16	3
24	female	19	128	NA	NA
26	female	25	137	NA	NA
28	female	29	199	9	1
29	male	25	135	4	0
30	male	18	110	13	2
33	female	24	112	NA	NA
35	female	19	101	4	5
36	female	22	45	0	1
41	male	27	124	NA	NA
43	male	19	44	6	2
44	female	36	35	2	0
45	male	22	58	5	0
52	female	22	NA	8	1
54	male	58	130	17	2
55	male	19	230	3	3
57	female	30	NA	10	1
58	female	27	110	11	4

Table 2.

Subject and sample characteristics of the neurosurgery study participants.

Sample tissues available for each individual varied. Details of analyzed samples are shown. Timing of when peripheral tissues were obtained are listed as "days". Day 0 is indicative of samples taken the day of surgery, after surgery is completed. For brain samples analyzed, the time (in minutes) between administration of the synthetic glucocorticoid and when the second (post) brain sample was resected was recorded. Abbreviations. GC: glucocorticoid, FCD: focal cortical dysplasia.

			Day(s) after surgery for peripheral sample collection							
Subject	**Sex**	**Age**	**Saliva**	**Blood**	**Buccal**	**Brain region (pre)**	**Brain region (post)**	**GC duration (minutes)**	**Pathology report (pre)**	**Pathology report (post)**
101	female	50	638							
102	female	46	309							
103	female	31	362	2						
104	female	51	263	0						
105	female	28	222	0						
106	male	61	2	0						
107	male	43	198	0						
108	male	40	241	0						
109	male	7	23	0	23	Occipital area	Occipital area	180	FCD type Ic and gliosis	Same as pre-GC sample
110	male	17	3	0	3	Inferior lateral temporal cortex	Inferior lateral temporal cortex	38	Evidence of cortical dysplasia, focal meningitis, and cortical injury consistent with electrode placement	Same as pre-GC sample
111	female	14	3	0	3					
112	male	31	2	0	2					
113	male	36	2	0	2	Temporal cortex	Temporal cortex	100	Moderate, patchy gliosis, and multiple superficial subacute microinfarcts with adjacent leptomeningeal chronic inflammation consistent with history of previous dural grid placement	Same as pre-GC sample
114	male	30	3	0	3	Lateral temporal cortex	Lateral temporal cortex	130	Patchy mild gliosis	Same as pre-GC sample

			Day(s) after surgery for peripheral sample collection							
Subject	**Sex**	**Age**	**Saliva**	**Blood**	**Buccal**	**Brain region (pre)**	**Brain region (post)**	**GC duration (minutes)**	**Pathology report (pre)**	**Pathology report (post)**
115	male	24	2	0	2	Lateral temporal cortex	Hippocampus	90	Mild neuronal heterotopia and mild patchy gliosis	Hippocampal sclerosis and moderate patchy gliosis
116	male	34	2	0	2	Lateral temporal cortex	Lateral temporal cortex	130	Patchy moderate gliosis and focal meningitis secondary to electrode placement	Same as pre-GC sample
117	female	49	2	0	2	Lateral temporal cortex	Inferior lateral temporal cortex	100	FCD type Ic and white matter gliosis	Subpial and white matter gliosis
118	male	5	4	0	4					
119	male	38	2	0	2	Lateral temporal cortex	Hippocampus	64	White matter and cortical gliosis and subacute infarct	Hippocampal sclerosis
120	male	11	122	0	14	Temporal operculum	Temporal operculum	152	Mild neuronal disorganization with white matter and cortical gliosis	Same as pre-GC sample
121	male	5	4	0	4	Temporal pole	Temporal pole	32	FCD	Same as pre-GC sample

Table 3.

Top differentially methylated CpGs associated with dexamethasone exposure in buccal samples from the dental procedure cohort.

cgid	Chr	Start	mean.Pre	mean.Post	mean.diff	p.val	Gene	Genic Location	Distance in bps
cg14315118	chr10	31,426,697	0.68	0.54	−0.14	3.21E-09	ZNF438	upstream	105,831
cg01119284	chr7	129,735,795	0.85	0.74	−0.10	1.11E-08	KLHDC10	intron	NA
cg26879918	chr15	78,617,501	0.82	0.70	−0.13	1.56E-08	miR-544; CRABP1	upstream; upstream	3,183; 23,071
cg16495532	chr1	101,472,258	0.73	0.58	−0.15	3.66E-08	DPH5	intron	NA
cg06335209	chr13	52,247,531	0.92	0.81	−0.10	4.36E-08	WDFY2	intron	NA

Table 4.

Methylation of pre- and post-dexamethasone buccal samples of CpGs within candidate genes from psychiatric disorders which were significant at FDR-adjusted p-value < 0.05 and with differences greater than 10% before and after dexamethasone exposure in the dental procedure cohort.

cgid	Chr	Start	mean.Post	mean.Pre	mean.diff	p.val	fdr	Gene
cg05039098	chr6	35,618,763	0.72	0.83	−0.11	1.16E-03	0.02	FKBP5
cg07696519	chr6	35,619,165	0.61	0.76	−0.15	8.77E-05	0.01	FKBP5
cg03546163	chr6	35,654,363	0.65	0.76	−0.11	4.73E-03	0.05	FKBP5
cg25708981	chr5	142,697,868	0.68	0.81	−0.13	1.77E-04	0.01	NR3C1
cg05900547	chr5	142,769,791	0.71	0.84	−0.13	3.39E-05	0.01	NR3C1
cg19432243	chr5	142,770,757	0.55	0.69	−0.14	2.95E-04	0.01	NR3C1
cg19820298	chr5	142,770,782	0.55	0.71	−0.16	3.96E-04	0.01	NR3C1
cg21979215	chr5	142,815,807	0.60	0.74	−0.14	2.85E-03	0.04	NR3C1
cg23143371	chr11	27,682,015	0.59	0.72	−0.13	3.59E-03	0.05	BDNF

Table 5.

Significance of top KEGG-defined pathways enriched for genes with CpGs differentially methylated after dexamethasone exposure in buccal samples from individuals who underwent tooth extraction.

N = number of genes in the pathway. DM = number of genes with differentially methylated CpGs within the associated pathway.

Pathway	N	DM	p.val	fdr
Olfactory transduction	406	50	3.50E-06	1.20E-03
Pentose and glucuronate interconversions	34	14	3.40E-05	4.30E-03
Ascorbate and aldarate metabolism	27	12	4.60E-05	4.30E-03
Steroid hormone biosynthesis	59	18	5.30E-05	4.30E-03
Retinol metabolism	66	15	2.00E-03	0.11
Drug metabolism - cytochrome P450	69	15	2.00E-03	0.11
Porphyrin and chlorophyll metabolism	42	12	2.80E-03	0.13
Axon guidance	174	46	5.20E-03	0.2
Chemical carcinogenesis	82	16	5.60E-03	0.2
Drug metabolism - other enzymes	79	17	6.70E-03	0.22