Journal of Psychiatric Research 83 (2016) 184–194

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Epigenetic signatures of childhood abuse and neglect: Implications for psychiatric vulnerability

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ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form
2 September 2016
Accepted 8 September 2016

Keywords:
DNA Methylation
Epigenome-wide
Child abuse
Neglect
Maltreatment
Stress

ABSTRACT

Childhood maltreatment is a key risk factor for poor mental and physical health. Recently, variation in epigenetic processes, such as DNA methylation, has emerged as a potential pathway mediating this association; yet, the extent to which different forms of maltreatment may be characterized by unique vs shared epigenetic signatures is currently unknown. In this study, we quantified DNA methylation across the genome in buccal epithelial cell samples from a high-risk sample of inner-city youth (n = 124; age = 16–24; 53% female), 68% of whom reported experiencing at least one form of maltreatment while growing up. Our analyses aimed to identify methylomic variation associated with exposure to five major types of childhood maltreatment. We found that: (i) maltreatment types differ in the extent to which they associate with methylomic variation, with physical exposures showing the strongest associations; (ii) many of the identified loci are annotated to genes previously implicated in stress-related outcomes, including psychiatric and physical disorders (e.g. *GABBR1, GRIN2D, CACNA2D4, PSEN2)*; and (iii) based on gene ontology analyses, maltreatment types not only show *unique* methylation patterns enriched for specific biological processes (e.g. physical abuse and cardiovascular function), but also share a '*common*' epigenetic signature enriched for biological processes related to neural development and organismal growth. A stringent set of sensitivity analyses were also run to identify high-confidence associations. Together, findings lend novel insights into epigenetic signatures of childhood abuse and neglect, point to novel potential biomarkers for future investigation and support a molecular link between maltreatment and poor health outcomes. Nevertheless, it will be important in future to replicate findings, as the use of cross-sectional data and high rates of polyvictimization in our study make it difficult to fully disentangle the shared vs unique epigenetic signatures of maltreatment types. Furthermore, studies will be needed to test the role of potential moderators in the identified associations, including age of onset and chronicity of maltreatment exposure.

1. Introduction

Childhood maltreatment, encompassing abuse and neglect, is a major public health concern that continues to affect up to one in four children worldwide, with often devastating developmental consequences (WHO, 2014). Children who experience maltreatment are at increased risk for a range of psychiatric problems, including anxiety, depression, post-traumatic stress, and antisocial behaviour (Cicchetti and Toth, 2005). The effects of maltreatment can extend well into adulthood, compromising relationship quality, economic productivity and physical health (Danese et al., 2009).

The theory of latent vulnerability proposes that maltreatment exposure calibrates a range of biological and neurocognitive systems in line with a threatening and unpredictable early environment (McCrory and Viding, 2015). While potentially adaptive in the short term, such changes can increase vulnerability in the long

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http://dx.doi.org/10.1016/j.jpsychires.2016.09.010
0022-3956/

term. Consistent with this view, numerous biological correlates of maltreatment have now been identified, including accelerated cellular ageing, neuroendocrine dysregulation, heightened inflammatory response as well as altered brain structure and function (Danese et al., 2011; McCrory et al., 2012; Shalev et al., 2013). Recent evidence indicates that, as well as affecting common biological pathways, different forms of maltreatment may also exert unique effects. For example, while abuse has been associated with changes in neural circuitry underlying threat processing, neglect has been associated with biological adaptations to low-complexity environments (Sheridan and McLaughlin, 2014).

A key challenge for current research is to understand how, at a molecular level, these environmental exposures are translated into phenotypic variation. Epigenetic processes, such as DNA methylation (DNAm), which control the functional regulation of gene expression are of particular interest in this regard, as mounting evidence suggests they can be modified by environmental factors (Jaenisch and Bird, 2003). For example, animal studies have found that a number of environmental stressors, such as poor maternal care, induce stable alterations in DNAm in the regulatory regions of several HPA axis genes (e.g. the glucocorticoid receptor), which in turn influence responses to future stressors (Turecki and Meaney, 2016). Similarly, a small number of human studies have documented a link between childhood maltreatment and aberrant DNAm in genes important for stress-response, immune function and neurodevelopment (Lutz and Turecki, 2014). DNAm has also been shown to regulate a wide range of neurobiological processes, including neurogenesis, synaptic plasticity, learning and memory (Baker-Andresen et al., 2013; Day et al., 2013) and aberrations in DNAm have been observed in a range of diseased states, including stress-related psychiatric disorders such as post-traumatic stress and major depression (Bergman and Cedar, 2013; Klengel et al., 2014).

To date, most epigenetic studies of maltreatment have focused on variation in the vicinity of a limited set of pre-selected candidate genes (i.e. *GR, FKBP5, BDNF and 5-HTT*) (Lutz and Turecki, 2014). As such, little is known about the broader effect of maltreatment on DNAm across the genome. This is a substantial limitation in light of the fact that maltreatment impacts multiple aspects of functioning, across psychological, physical, and social domains. Furthermore, existing studies have primarily examined global maltreatment (Labonte et al., 2012; Prados et al., 2015; Suderman et al., 2014; Yang et al., 2013), so that the extent to which different maltreatment types may have common vs distinct epigenetic signatures is unclear. To address these outstanding questions, we explored the relationship between DNAm and five types of maltreatment in a sample of high-risk youth, using genome-wide DNAm data drawn from buccal epithelial cells.

2. Methods and materials

2.1. Participants

The current sample was recruited as part of a larger study examining the effects of developmental adversity on individual functioning ($n = 204$, age range = 16–24 years). Analyses only included participants for whom DNAm data was available ($n = 124$). Youth from deprived inner London areas were recruited through multiple channels including inner-city colleges, internet websites and a charity providing services and support to self-referred youth. The sample was 53% female and ethnically diverse (49% White, 33% Black, 18% other). The study was carried out in accordance with the latest version of the Declaration of Helsinki. The study design was reviewed and approved by the UCL Research Ethics Committee (*ID No*: 2462/001) and all participants provided informed consent prior to participation, after the nature of the procedures had been fully explained. Further details of the sample and recruitment procedures are available elsewhere (Cecil et al., 2014).

2.2. Measures

Childhood maltreatment – Childhood maltreatment was assessed using the 28-item, self-report Childhood Trauma Questionnaire (CTQ; Bernstein and Fink, 1998). The CTQ screens for experiences of maltreatment "while growing up" and comprises of 5 continuous subscales: emotional abuse, sexual abuse, physical abuse, emotional neglect and physical neglect. The scales show high internal consistency in our sample ($\alpha = 0.70–0.97$). For descriptive purposes only, we also classified participants as having experienced maltreatment (i.e. yes/no) if they scored above the 'Low' threshold specified by the CTQ manual for at least one maltreatment type. By including 'I currently feel unsafe at home' as an additional yes/no item we were able to ascertain that none of the participants in the study were currently vulnerable to violence in the domestic environment (e.g. by family or partner). As such, the present study investigates the effects of childhood (i.e. past) maltreatment.

DNA methylation – DNA was extracted from buccal epithelial cells using procedures described in Freeman et al. (2003). 500 ng of high molecular weight DNA was subjected to sodium bisulfite conversion using the EZ-DNA methylation kit (Zymo Research, Orange, CA, USA) using the manufacturers standard protocol. DNAm was quantified using the Illumina HumanMethylation450 BeadChip (Illumina, USA) with arrays scanned using an Illumina iScan (software version 3.3.28). The Illumina 450 K array interrogates >485,000 probes covering 99% of Reference Sequence (RefSeq) genes, with an average of 17 CpG sites per gene region. As the samples were run in a single batch, there was no need for batch correction. To account for potential chip and position effects, we randomized sample chip allocation and placement on the chip. Initial data quality control was conducted using GenomeStudio (version 2011.1) to determine the status of staining, extension, hybridization, target removal, bisulfite conversion, specificity, non-polymorphic and negative controls. Samples that survived this stage were checked for concordance between their reported and assessed sex and then quantile normalised using the *dasen* function within the *wateRmelon* package (wateRmelon_1.0.3; Pidsley et al., 2013) in R. Probes were removed if they were cross-reactive, polymorphic, used for sample identification on the array, had a SNP at the single base extension with a minor allele frequency larger than 5% (i.e. common polymorphisms) or were located on the Y chromosome, leaving a total of 413,239 probes (Chen et al., 2013; Price et al., 2013). DNAm levels are indexed by beta values (ratio of methylated signal divided by the sum of the methylated and unmethylated signal, M/M+U).

2.3. Data analysis

All analyses were performed within the R statistical environment (version 3.0.1). Methylation data was regressed for sex, age and self-reported ethnicity to account for potential confounding effects (Liang and Cookson, 2014). The analysis proceeded in three steps. First, we ran five independent epigenome-wide association analyses – one for each maltreatment type measured – using linear regression models. Probes were considered significant if they survived a False Discovery Rate (FDR) correction of $q < 0.05$. Only maltreatment types that were associated with at least one FDR-corrected probe were carried forward to the next step. Second, we identified which probes were most consistently associated with *all* types of maltreatment, by ranking them in order of average