Review

Biological Psychiatry

Effects of the Social Environment and Stress on Glucocorticoid Receptor Gene Methylation: A Systematic Review

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ABSTRACT

The early-life social environment can induce stable changes that influence neurodevelopment and mental health. Research focused on early-life adversity revealed that early-life experiences have a persistent impact on gene expression and behavior through epigenetic mechanisms. The hypothalamus-pituitary-adrenal axis is sensitive to changes in the early-life environment that associate with DNA methylation of a neuron-specific exon 1_7 promoter of the glucocorticoid receptor (GR) (*Nr3c1*). Since initial findings were published in 2004, numerous reports have investigated GR gene methylation in relationship to early-life experience, parental stress, and psychopathology. We conducted a systematic review of this growing literature, which identified 40 articles (13 animal and 27 human studies) published since 2004. The majority of these examined the GR exon variant 1_F in humans or the $GR1_7$ in rats, and 89% of human studies and 70% of animal studies of early-life adversity reported increased methylation at this exon variant. All the studies investigating exon $1_F/1_7$ methylation in conditions of parental stress (one animal study and seven human studies) also reported increased methylation. Studies examining psychosocial stress and psychopathology had less consistent results, with 67% of animal studies reporting increased exon 1_7 methylation and 17% of human studies reporting increased exon 1_F methylation. We found great consistency among studies investigating early-life adversity and the effect of parental stress, even if the precise phenotype and measures of social environment adversity varied among studies. These results are encouraging and warrant further investigation to better understand correlates and characteristics of these associations.

Keywords: DNA methylation, Early-life adversity, Epigenetics, Glucocorticoid receptor, Social environment, Systematic review

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There is substantial theoretical and empirical research supporting an association between early-life environmental adversity and poor lifetime mental health outcomes (1–12). A critical issue concerns the molecular mechanisms that account for such strong and long-lasting effects. There is evidence suggesting that early-life environmental influences induce changes in stable epigenetic states that regulate gene expression and ultimately complex neural functions. Thus, in both rodents and nonhuman primates, the early-life environment, including the quality of maternal care, regulates hypothalamus-pituitary-adrenal (HPA) axis function in adulthood (13–15). Variations in the early social environment in rodents, modeled by maternal care, reveal profound and persistent alterations in gene expression and behavior that are mediated through epigenetic mechanisms, including changes in DNA methylation (16). The offspring of mothers with an increased frequency of pup licking/grooming (LG) (i.e., high LG mothers) show increased hippocampal glucocorticoid receptor (GR) (*Nr3c1*) expression, greater negative feedback regulation over hypothalamic corticotropin releasing factor (CRF), and more modest responses to stress compared with the offspring of low LG mothers (16–18). Variations in maternal LG are linked to an epigenetic modification of a neuron-specific exon 1_7 GR promoter (16) such that increased maternal LG associates with decreased methylation of the exon 1_7 promoter and increased hippocampal GR expression.

Subsequent studies in humans have expanded on the findings in rats. Accordingly, evidence for a long-term effect of early-life adversity (ELA) on the epigenetic state of the human genome was observed while investigating the methylation state of the GR gene in the hippocampus of individuals who died by suicide and had histories of child abuse (19). ELA in humans reprograms the DNA methylation patterns of the GR gene exon 1_F ($GR1_F$; $GR1_7$ homologue in rats) promoter and decreases $GR1_F$ expression in the hippocampus of suicide completers with a history of child abuse compared with nonabused suicide completers and healthy control subjects (19). An earlier study reported that children born to mothers with depression, irrespective of selective serotonin reuptake inhibitor use, exhibited higher $GR1_F$ methylation levels (20). Since these first reports, several studies have investigated the effect of environmental adversity, measured by ELA or exposure to parental stress, on GR gene methylation, using both animal models and human samples. These studies also used

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different designs, measures of adversity, and tissue samples and investigated methylation of diverse GR gene sequences. A growing number of studies have also been investigating the relationship between psychological stress or psychopathology and GR methylation. We conducted a systematic review of the growing literature investigating the relationship between environmental experience, stress, and GR gene methylation.

METHODS AND MATERIALS

Study Identification

We performed a search of association studies of the GR gene and DNA methylation. The primary search was carried out through the National Library of Medicine PubMed and a replication search was conducted through the Web of Knowledge database. The search included publications from 2004 up to July 2014 using the Weaver *et al.* (16) study as a starting point. The Medical Subject Headings terms used were "glucocorticoid receptor" or NR3C1 and epigenetics or "DNA methylation." Additional articles were found by scanning the list of references of the original publications and review articles. Only articles in English and those investigating humans or other mammals were included.

Study Selection

The studies included in this systematic review met the following criteria: 1) use of a case-control or cohort design; 2) use of at least one analysis investigating DNA methylation of the GR gene in response to a change or perturbation in social environment; and 3) inclusion of studies independent from one another. Analyses based on the same set of data were excluded. In such cases, only the larger or more representative sample was retained. Studies in which a control group was absent also were not included.

Data Extraction

Information for each study was extracted based on nine variables: 1) species (human or nonhuman); 2) study (experimental) group; 3) sex; 4) sample size; 5) methodology (DNA methylation assessment); 6) tissue(s) investigated; 7) subject age at tissue collection; 8) region or first exon variant(s) investigated; and 9) effect on methylation (Tables S1–3). Studies were then grouped according to a broad classification of the study criteria and attributed to Tables S1, S2, or S3. Within each table, animal- and human-based studies were considered independently.

RESULTS

There is a growing number of studies reporting changes in GR gene methylation in association with social environment and stress. Our search identified a total of 430 articles. Of these, 173 were review papers and were excluded. Another 210 articles were excluded due to lack of relevance to the topic of this review. Seven studies were removed because they were not independent, as they investigated samples for which results had been reported elsewhere. In all, 40 articles met all the specified criteria. These were then sorted based on whether they addressed GR gene methylation changes in response to ELA (22 articles; Table S1), parental stress (9 articles; Table S2), or psychological stress/psychopathology (11 articles; Table S3) [two studies were included in two tables because they addressed both ELA and psychological stress (21,22)]. Within each table, the articles were further subdivided into animal studies, human studies using peripheral tissues, and human studies using central nervous system (CNS) tissue.

Sample Type: Species and Tissues Studied

Among the animal studies included, all used either rat (8 of 13 studies) or mouse (5 of 13 studies). All animal studies examined brain tissue, and one study compared brain tissue and fecal matter (23), while another compared brain tissue and adrenal tissue (24). The brain region studied varied, including cortical and subcortical regions.

In the human studies, the majority of articles reported on GR gene methylation in peripheral tissues, where the term peripheral refers to tissues other than the CNS. In the human peripheral studies category, 20 of the 24 articles used blood tissue, while 2 used saliva (25,26), 1 used buccal epithelial cells (27), and 1 used placental tissue (28). Of the 3 studies examining brain tissue (the human central tissues category), all examined tissues from the limbic and cortical regions (19,29,30) and focused in particular on the hippocampus. In addition, Labonté *et al.* (29) examined the anterior cingulate cortex, while Alt *et al.* (30) investigated the amygdala, inferior prefrontal gyrus, cingulate gyrus, and nucleus accumbens.

GR Gene Region Examined. The GR gene in humans and rodents consists of 11 exons including untranslated first exon variants (31). Nine untranslated first exon variants, each possessing their own promoter region, have been identified in humans ($1_{A,\ I,\ D,\ J,\ E,\ B,\ F,\ C,\ \text{and}\ H}$) and in rats ($1_{1,\ 4–11}$). We found significant heterogeneity in the reporting and identification of the specific regions of the first exon variants that were studied. Specifically, there was no consistency in how CpG sites were identified and labeled, making the determination of overlapping regions difficult. This made detecting consistency in findings at the sequence level among studies challenging. We compiled the sequence data that we were able to retrieve from information published in all the human studies and located the regions within the GR gene containing the first exon variants investigated (Figure S1 in Supplement 1).

Early-Life Adversity

Experimental Groups. To ensure the maximum impact of the review and allow for the comparison of results, we carefully selected articles that used similar criteria to define ELA.

In animal models, early-life experience was characterized by the use of maternal care models in six studies (16,23,32–35) or maternal separation models in four studies (22,36–38).

Human studies defined ELA as exposure to traumatic events in childhood, including emotional, physical, or sexual abuse; neglect; early parental death; and other traumatic events. All studies included subjects who had been exposed to childhood abuse, with variations in the type of abuse included. In addition, three studies included early parental death as a marker of ELA (25,39,40).