Differential Glucocorticoid Receptor Exon 1_B, 1_C, and 1_H Expression and Methylation in Suicide Completers with a History of Childhood Abuse

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Background: Childhood abuse alters hypothalamic-pituitary-adrenal (HPA) function and increases the risk of suicide. Hippocampal glucocorticoid receptor (GR) activation regulates HPA activity, and human GR expression (hGR) is reduced in the hippocampus of suicide completers with a history of childhood abuse compared with controls. The abuse-related decrease in hGR expression associates with increased DNA methylation of the promoter of the hGR_{1F} variant in the hippocampus.

Methods: In this study, we investigated the expression and methylation levels of other hGR splice variants in the hippocampus and anterior cingulate gyrus in suicide completers with and without a history of childhood abuse and in controls. Expression levels were quantified using quantitative reverse-transcriptase polymerase chain reaction and promoter methylation was assessed by pyrosequencing.

Results: In the hippocampus, the expression of total hGR and variants 1_B, 1_C, and 1_H was decreased in suicide completers with histories of abuse compared with suicides with no histories of abuse and with control subjects. In the anterior cingulate gyrus, however, no group differences in hGR total or variant expression were found. Site-specific methylation in $hGR1_B$ and 1_C promoter sequences were negatively correlated with total hGR messenger RNA, as well as with $hGR1_B$ and 1_C expression. Luciferase assay showed that methylation in hGR promoter decreases transcriptional activity. In contrast, total and site-specific methylation in the $hGR1_H$ promoter was positively correlated with total hGR messenger RNA and $hGR1_H$ expression.

Conclusion: These findings suggest that early-life events alter the expression of several hGR variants in the hippocampus of suicide completers through effects on promoter DNA methylation.

Key Words: Childhood abuse, DNA methylation, early-life stress, epigenetics, gene expression, glucocorticoid receptor (GR), HPA axis, suicide

Suicide can occur as a result of the interaction of multiple influences (1–4), including childhood sexual and physical abuse (5–8). There is substantial evidence for a link between suicide and hypothalamic-pituitary-adrenal axis (HPA) hyperactivity (9), which in turn, associates with early-life adversity (ELA) (10). Compared to nonabused control subjects, abuse victims with major depressive disorder exhibit higher plasma levels of corticotropin (ACTH) and cortisol following dexamethasone challenge and Trier social stress test (11,12). In addition, results from animal studies suggest that ELA modifies the functioning of the HPA axis and induces behavioral alterations during adulthood (13,14). Taken together, these findings suggest that alterations in HPA activity induced by ELA may influence the risk for suicide.

The glucocorticoid receptor (GR) regulates HPA axis and stress reactivity (10) and GR expression in brain is stably influenced by early experience (4). Variations in quality of the postnatal environment can influence DNA methylation level of specific gene promoters, including that for the GR (13–18). DNA methylation is a covalent modification of cytosines in DNA that regulates genome function. DNA methylation in promoters and gene regulatory regions associates with silencing of gene expression (19). For example, in rats, adult offspring of low licking and grooming mothers show decreased hippocampal $GR1_7$ expression associated with overall promoter hypermethylation that constrains the binding of the transcription factor nerve growth factor-induced protein A (NGFI-A) (14). Translational studies in humans (17) show decreased hippocampal $hGR1_F$ (hGR) expression, the human $GR1_7$ homologue in rats, in abused suicide completers compared with nonabused suicide and control subjects. The decrease in $hGR1_F$ expression associates with reduced total hGR messenger RNA and with site-specific promoter hypermethylation in a region that contains an NGFI-A binding site (17). Such patterns of methylation repress NGFI-A binding to $hGR1_F$ promoter and decrease transcriptional activity in reporter assays. These results suggest that site-specific hypermethylation in the $hGR1_F$ promoter associates with reduced GR expression in the brain of abused, suicide completers (17).

The human hGR gene is located on chromosome 5 at locus q31–q32 and is composed of eight coding exons preceded by a number of noncoding first exons (20,21). Each of these first exons contains a transcription start site (TSS) preceded by a promoter (22,23). Because the ATG start codon is located only at the beginning of exon 2, first exons remain untranslated, although it is suggested that they may be involved in the determination of hGR expression in various tissues (21,24). Seven (D, J, E, B, F, C, and H) of these first exons are found within a CpG island in a region encompassing 4.5 kb upstream of exon 2. First exon variant expression differs across tissues (21). The 1_C and 1_B variants are the most expressed first exons in the hippocampus followed by 1_F and 1_H and finally by 1_D, 1_J and 1_E (25). The genomic location of these exons within a CpG island renders them susceptible to epigenetic regulation. For instance, numerous transcription factor binding sites lie within the noncoding exon 1 promoters (17,26–28). Moreover,

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methylation in the promoter of these exons may regulate differential hGR transcript expression, and there is considerable interindividual heterogeneity in methylation levels of hGR promoters (29).

Although there is increased methylation of the $hGR1_F$ promoter in the hippocampus of abused suicide completers, it remains unclear whether methylation of the promoters of the other noncoding exon 1 variants is also altered by ELA. In this study, we investigated the association of ELA with methylation in the promoters of hGR's noncoding exon 1 variants and the relationship with expression of these transcripts. This study focused on three noncoding exons with the highest expression in the hippocampus besides 1_F namely, $hGR1_B$, 1_C, and 1_H. $hGR1_F$ was not included in this study because it was already assessed in a previous study by our group (17). In addition to the hippocampus, we investigated the anterior cingulate cortex (ACC), a brain region implicated in mood regulation (30). Promoter DNA methylation was assessed in regions predicted to be enriched in transcription factor binding sites. Our results suggest that $hGR1_B$, 1_C, and 1_H expression in the hippocampus, but not in ACC, of abused suicide completers is decreased. This effect was associated with site-specific DNA methylation alterations, and luciferase assay showed that methylation in hGR promoter decreases transcriptional activity. These data support the hypothesis that child abuse is associated with a coordinated DNA methylation response in multiple promoters of the hGR gene contributing to decreased hGR expression and HPA dysregulation.

Methods and Materials

Complete methods are described in Supplement 1.

Sample Selection

Brain tissue was obtained from the Quebec Suicide Brain Bank (Douglas Mental Health University Institute, Verdun, Quebec). All subjects were male Caucasians of French-Canadian descent, and groups (abused suicides [SA], nonabused suicides [SNA], controls [CTRL]) were matched for age, pH, and postmortem intervals (PMI; Table S1 in Supplement 1). The control group was composed of psychiatrically healthy individuals who died by accidental causes with no history of child abuse. Presence of severe ELA was assessed based on adapted Childhood Experience of Care and Abuse interviews assessing various dimensions of childhood experience including experiences of abuse (31). Hippocampus ($n = 56$; 21 SA, 21 SNA, and 14 CTRL) and Brodmann area 24 ($n = 50$; 22 SA, 14 SNA, and 14 CTRL) were carefully dissected by experienced histopathologists using reference neuroanatomical maps (32,33).

Quantification of Gene Expression Using Reverse-Transcriptase Polymerase Chain Reaction

Three reverse transcription reactions were performed independently using primers targeting specifically NR3C1 (glucocorticoid receptor, hGR), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and β-Actin. The quantity of complementary DNA was extrapolated from a standard curve, composed of a mix of complementary DNA from all subjects and including six dots, each of which was four times diluted (4:1). Mean quantities from all sample replicates were normalized to the reference gene GAPDH and β-Actin, averaged together and analyzed by mean of one-way analysis of variance followed by Fisher's least significant difference post hoc test.

Methylation Analysis

DNA was extracted and sodium bisulfite converted according to the manufacturer's instructions (Qiagen, Germantown, Maryland). Primers and sensitivity assays for pyrosequencing targeting three regions of the hGR variant promoters were designed and optimized by EpigenDx (Worcester, Massachusetts; Tables S3 and S4 in Supplement 1). Pyrosequencing runs were performed by EpigenDx. Mean percentage of methylation at each CpG site, and at all sites (global methylation; Figure 1) was compared across groups and analyzed by two way-mixed model analysis of variance with groups as a fixed factor and CpGs as a repeated measure followed by least significant difference post hoc tests.

Luciferase Assays

$hGR1_C$ full length promoter was amplified by polymerase chain reaction from human genomic DNA. $hGR1_C$ promoter inserted into pGL3 plasmid was methylated by patch methylation before transfection into Be(2)c cells. Firefly renilla plasmid was used as a control for transfection efficiency and to normalize luciferase activity. All experiments were performed in seven replicates. Results were analyzed by *t* test.

Results

hGR Expression in the Hippocampus (HPC) and ACC in Abused Suicide Completers

The expression of hGR total and the first noncoding exons 1_B, 1_C, and 1_H was compared in the hippocampus and ACC of SA, SNA, and normal control subjects. There was no significant difference across groups for age, pH, PMI, RNA integrity values, and psychiatric medication prescription status (Table S1 in Supplement 1), and analyses of covariance revealed no significant effect of these covariables on gene expression results. The results reveal that relative expression levels of hGR total and first noncoding exons were decreased in SA compared with SNA and CTRL independently of the effect of covariables (Figure 2). Indeed, we found a significant group effect for the mean expression levels of hGR total [$F(2,47) = 10.49$, $p < .001$; Figure 2A]. Post hoc analyses revealed significantly decreased hGR total expression in SA compared with CTRL ($p < .005$) and SNA ($p < .001$), thus replicating our earlier finding (17). A significant group effect was found for the mean expression levels of $hGR1_B$ [$F(2,48) = 5.23$, $p < .01$; Figure 2B). Post hoc tests revealed a significant decreased expression in samples from SA compared with those from SNA ($p < .05$) and CTRL ($p < .005$), with no difference between SNA and CTRL values. A significant group effect was also found for the relative expression of $hGR1_C$ [$F(2,46) = 5.82$, $p < .01$; Figure 2C]. Post hoc tests showed that $hGR1_C$ relative expression is significantly decreased in SA compared with SNA ($p < .005$) and CTRLs ($p < .05$) with no difference between SNA and CTRL. Finally, we found a significant group effect for $hGR1_H$ relative expression [$F(2,45) = 4.29$, $p > .05$; Figure 2D] with post hoc test revealing significantly decreased relative expression in SA compared to SNA ($p < .01$) and CTRL ($p < .05$). No significant differences in expression were observed between SNA and CTRL.

In contrast to the hippocampus, analysis of samples from the ACC showed no difference in hGR total relative messenger RNA expression across SA, SNA, and CTRL [hGR total: $F(2,44) = .49$, $p > .1$; Figure 2E]. In addition, no significant difference was found across groups in the ACC relative expression of any of the first noncoding exons [$hGR1_B$ $F(2,44) = .06$, $p > .1$], 1_C [$F(2,46) = .47$, $p < .1$] and 1_H [$F(2,43) = .66$, $p > .1$; Figure 2F–2H].

Differential DNA Methylation in Abused Suicide Completers

DNA methylation regulates hippocampal hGR expression, and increased methylation associates with a downregulation of $hGR1_F$ expression in abused suicide completers (17,19). We therefore measured DNA methylation levels across the promoter sequences of differentially expressed nontranslated exon 1 variants. We focused