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DNA methylation differences at the glucocorticoid receptor gene in depression are related to functional alterations in hypothalamic–pituitary–adrenal axis activity and to early life emotional abuse

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ABSTRACT

Depression is associated with alterations in hypothalamic–pituitary–adrenal (HPA) axis activity. A proposed mechanism to explain these alterations are changes in DNA methylation levels, secondary to early life adversity (ELA), at stress-related genes. Two gene regions that have been implicated in the literature, the glucocorticoid receptor gene (*NR3C1*) exon 1F and the *FKBP5* gene intron 7 were examined in 67 individuals (33 depressed patients and 34 controls). We investigated whether cortisol concentrations, evaluated in 25 depressed patients and 20 controls, and measures of ELA were associated with the degree of methylation at these candidate gene regions. Mean *NR3C1* exon 1F DNA methylation levels were significantly increased in the depressed cohort and the degree of methylation was found to be positively associated with morning cortisol concentrations. DNA methylation levels at specific CG sites within the *NR3C1* exon 1F were related to childhood emotional abuse severity. DNA methylation at CG38 was related to both HPA axis and childhood emotional abuse measures in the depressed group. No *FKBP5* differences were revealed. Our findings suggest that hypermethylation at the *NR3C1* exon 1F may occur in depression. This locus-specific epigenetic change is associated with higher basal HPA axis activity, possibly reflecting acquired glucocorticoid receptor resistance.

1. Introduction

Major depressive disorder (MDD) is a debilitating mood disorder with a lifetime prevalence rate of approximately 17% (Kessler et al., 2005). Despite extensive research, there are no established biological markers for the diagnosis of depression (Mossner et al., 2007). Longitudinal studies indicate that many adulthood-onset diseases are associated with adverse developmental or biological events experienced early in life. The latency between exposure to adversity and development of a disorder can span years, and even decades in some instances (Shonkoff et al., 2009). Exposure to early life adversity (ELA) has been identified as a major risk factor in the development of almost all psychiatric disorders (Kessler et al., 2010), including MDD (Kessler and Magee, 1993). It has been reported that adult patients with MDD have a two-fold higher rate of ELA compared to healthy controls and that ELA has been shown to predict earlier-onset depression, a higher risk of recurrent depression, more severe course of illness, greater chronicity and suicide (Williams et al., 2016). MDD patients with a history of ELA show different treatment responses reinforcing the idea of diverse pathways to the loose clinical syndrome of depression, one of which is

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ELA (Nemeroff et al., 2003).

The hypothalamic–pituitary–adrenal (HPA) axis is the main stress response system (Bellavance and Rivest, 2014). Through glucocorticoid receptors (GR) cortisol mediates a myriad of tissue-specific effects (Young et al., 2003). Importantly, cortisol signaling via GR is responsible for negative feedback inhibition of the HPA axis. A frequently replicated finding in depression research is the association of depression with hyperactivity of the HPA axis (Pariante and Lightman, 2008). It has been hypothesized that the increased HPA axis activity results from dysfunctional feedback because of reduced efficacy of central GR function (Young et al., 2003). The cortisol awakening response (CAR), the brisk increase of cortisol levels within 20–30 min of awakening in the morning (Fries et al., 2009), provides a naturalistic test for a cortisol stress response. Several studies have demonstrated an increased CAR in currently depressed individuals (Suzuki et al., 2013; Vreeburg et al., 2009), as well as in recovered patients with depression (Bhagwagar et al., 2003).

The GR co-chaperone protein FK506 binding protein 5 (FKBP5) is an immunophillin protein that accompanies the inactive GR complex in the cytoplasm. FKBP5 reduces the affinity of the GR complex for cortisol and thus decreases overall GR signaling (Rao et al., 2016). It is hypothesized that genetic variants within the *FKBP5* gene may influence individuals' susceptibility to depression, with certain variants associated with increased FKBP5 protein levels (Szczepankiewicz et al., 2014), and consequently with relative decrease in GR complex activity. A recent meta-analysis supports the association between specific FKBP5 genetic variants and an increased risk of depression (Rao et al., 2016).

Epigenetic mechanisms are essential for the regulation of gene expression as they can modulate events at the level of transcription and translation (Portela and Esteller, 2010). Epigenetic mechanisms may provide an etiological link between ELA and the syndrome of depression (Dalton et al., 2014). The addition of a methyl group to the cytosine within a CG site in a critical regulatory region can inhibit transcriptional activity (Farrell and O' Keane, 2016).

In relation to MDD and alterations in HPA axis activity, an interesting epigenetic modification is methylation of a nerve growth factor-inducible protein A (NGFI-A) binding site located in exon 1F of the GR gene, the nuclear receptor subfamily 3 group C member 1 (*NR3C1*) (Swirnoff and Milbrandt, 1995), that is associated with ELA. Alterations at or near this NGFI-A binding site may explain one of the biological links between ELA and the development of depressive illnesses (Smart et al., 2015). *NR3C1* exon 1F hypermethylation in relation to ELA has been demonstrated in peripheral tissues, including cord blood, white blood cells and whole blood (Oberlander et al., 2008; Perroud et al., 2011; Radtke et al., 2011,2015) and would potentially result in dysregulation of HPA axis negative feedback were it occurring in the brain. It is possible that this hypermethylation is indeed occurring centrally as increased DNA methylation has been reported in the hippocampus of the orthologue 1_7 site in stressed rats (Weaver et al., 2004) and in the hippocampus of abuse victims who died by suicide (McGowan et al., 2009).

FKBP5 has recently attracted attention as a protein possibly implicated in the area of epigenetic-mediated gene-childhood trauma interaction leading to adult mental illness. Klengel et al. performed the first human study examining gene-ELA interaction and methylation at *FKBP5*. They showed that individuals suffering from post-traumatic stress disorder (PTSD) who were exposed to ELA and who carried a functional *FKBP5* risk T allele at rs1360780 had decreased levels of methylation at intron 7 of the *FKBP5* gene. This intronic demethylation was associated with an increase in FKBP5 induction by GR activation, leading to symptoms of GR resistance (Klengel et al., 2013). One study examining *FKBP5* methylation in individuals with the risk T allele and remitted depression revealed a (non-significant) trend towards higher methylation levels compared to healthy controls (Höhne et al., 2015). There are a few more studies that examined FKBP5 methylation association to ELA but the direction of effect was inconsistent (Needham et al., 2015; Tyrka et al., 2015).

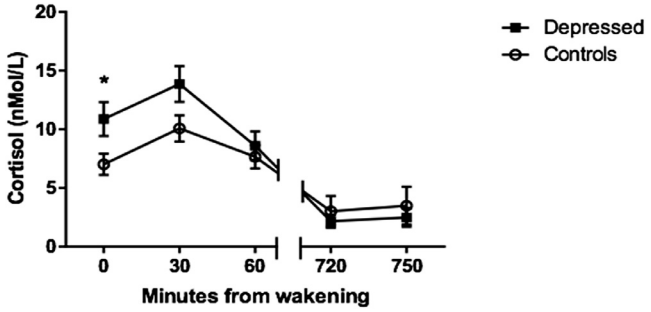

Fig. 1. Morning and evening cortisol concentrations in depressed patients and healthy controls. Cortisol concentrations at three morning and two evening time points in depressed patients and healthy controls. Data are expressed as mean cortisol concentrations (nMol/L) with SEM, analysis of variance, with BMI and education as covariates on log transformed data, *$p \leq 0.05$ vs. controls.

No studies, to our knowledge, have examined *NR3C1* and *FKBP5* methylation in the same individuals, and the possible associations between their DNA methylation levels and HPA axis function. The aims of this study were to determine if DNA methylation at the *NR3C1* exon 1F and/or *FKBP5* intron 7 were altered in individuals with depression compared to controls. We also explore possible relationships between *NR3C1* and *FKBP5* DNA methylation and HPA axis function as assessed through the CAR (Fig. 1).

2. Methods and materials

2.1. Patient recruitment and assessment

Depressed patients were recruited from the Tallaght Mental Health Services. Any patient presenting with depression was considered for the study. They were screened for eligibility by a consultant psychiatrist. Screening involved assessing whether patients fulfilled criteria for a Major Depressive Episode (MDE) from the Mini International Neuropsychiatric Interview (M.I.N.I) (Sheehan et al., 1997); scored > 17 on the Hamilton Depression Rating 21-Item Scale (HAM-D-21) (Hamilton, 1960), indicative of moderate depression; were not suffering from a psychotic or substance abuse disorder or from any chronic medical illnesses and were not taking any steroid medications, excluding the contraceptive pill. Healthy, control individuals were screened and recruited from the general population. Screening involved establishing that individuals had never experienced a mental health disorder, scored ≤ 7 on the HAM-D-21, were not suffering from any chronic medical illnesses, and were not taking any steroid medications, except the oral contraceptive pill. For both groups, 45 years, considered the beginning of middle age (Stevenson, 2010), was selected as the maximum age for inclusion to reduce the possibility of age-related effects on biological data. The groups were balanced for age and sex. If individuals met inclusion and exclusion criteria, they were invited to participate. If, following a thorough explanation of the study, and they remained willing to participate, they gave written informed consent. As this was not an intervention study, all treatment was continued as usual. Detailed demographic information was collected. The International Standard Classification of Education (ISCED) is a standardized scale used to gather information on educational achievement (UNESCO, 2011) and was used to grade participants' level of education. Participants were then administered a battery of clinical instruments: Centre for Epidemiological Studies Depression Rating Scale (CES-D) (Orme et al., 1986), suicidality module (Module C) of the M.I.N.I (Sheehan et al., 1997) and the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1997). The CTQ is a retrospective, self-report measure of ELA. The CTQ contains five subscales; three assessing abuse