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DNA methylation and sex-specific expression of *FKBP5* as correlates of one-month bedtime cortisol levels in healthy individuals

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ABSTRACT

Chronic exposure to cortisol is associated with cardiovascular, metabolic, and psychiatric disorders. Although cortisol can be readily measured from peripheral sources such as blood, urine, or saliva, multiple samplings spanning several days to weeks are necessary to reliably assess chronic cortisol exposure levels (referred to as cortisol load). Although cortisol levels in hair have been proposed as a measure of cortisol load, measurement is cumbersome and many people are not candidates due to short hair length and use of hair dyes. To date, there are no blood biomarkers that capture cortisol load.

To identify a blood biomarker capable of integrating one-month cortisol exposure levels, 75 healthy participants provided 30+ days of awakening and bedtime saliva cortisol and completed psychosocial measures of anxiety, depression, and stress. Mean daily awakening and bedtime cortisol levels were then compared to CpG methylation levels, gene expression, and genotypes of the stress response gene *FKBP5* obtained from blood drawn on the last day of the study.

We found a correlation between *FKBP5* methylation levels and mean 30+day awakening and bedtime cortisol levels ($|r| \geq 0.32$, $p \leq 0.006$). We also observed a sex-specific correlation between bedtime cortisol levels and *FKBP5* mRNA expression in female participants ($r = 0.42$, $p = 0.005$). Dividing the 30-day sampling period into four weekly bins showed that the correlations for both methylation and expression were not being driven by cortisol levels in the week preceding the blood draw. We also identified a female-specific association between *FKBP5* mRNA expression and scores on the Beck Anxiety Inventory ($r = 0.37$, $p = 0.013$) and Beck Depression Inventory-II ($r = 0.32$, $p = 0.033$). Finally, DNA was genotyped at four SNPs, and variation in rs4713902 was shown to have an effect on *FKBP5* expression under a codominant model ($f = 3.41$, $p = 0.048$) for females only.

Our results suggest that blood *FKBP5* DNA methylation and mRNA expression levels may be a useful marker for determining general or sex-specific 30-day cortisol load and justifies genome-wide approaches that can potentially identify additional cortisol markers with broader clinical utility.

1. Introduction

Allostatic load is a theoretical construct representing the sum of detrimental consequences of stress accumulated over time (Ogden, 2004). High allostatic load is a major contributor to poor health outcomes. Chronic exposure to the stress hormone cortisol is a significant component of allostatic load, and is associated with cardiovascular and metabolic disorders.(Bose et al., 2009; Brydon et al., 2006; Hackett and Steptoe, 2016; Kelly and Ismail, 2015) The brain is especially susceptible to cortisol, as prolonged exposure is associated with an increase in neuropsychiatric disorders,(Fardet et al., 2012) decrease in hippocampal volume,(Rahman et al., 2016) and cognitive decline.(Arnsten, 2015; McKlveen et al., 2016) Given the detrimental impact of chronic exposure to cortisol, an easily accessible biological measurement capable of capturing long term cortisol exposure would be immensely useful for testing the association of cortisol load and disease susceptibility.

While cortisol can be measured from various peripheral sources such as urine, blood, saliva, and hair, measurements from these sources either do not adequately reflect long-term cortisol exposure or are

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burdensome to perform. Lack of accuracy of these measurements is attributed to the pulsatile nature of cortisol secretion. Cortisol measured in urine, blood, and saliva tends to only reflect the most recent circadian and stressful events. While the assessment of 24-hour urine free cortisol levels provides a metric for a single day's cortisol exposure, other cortisol measures in urine, saliva, or blood provide even less time-based information. Unless multiple samplings are measured, an accurate determination of cortisol load is not possible. Although measurement of cortisol in hair samples is thought to indicate long-term exposure to cortisol, sampling is cumbersome and not always possible especially in individuals with short hair, recent hair shampooing and/or use of hair dyes.(Heinze et al., 2016; Rippe et al., 2016)

As a motivating example, the field of diabetes management was greatly advanced when it was determined that a single glycated hemoglobin level was a surrogate that integrated the previous 90 days of glucose exposure.(Saudek et al., 2008) A single blood test that integrates long-term exposure to cortisol would permit assessment of inter-individual differences in cortisol load thus providing a tool to predict future health consequences and treatment options.

Although blood cortisol measurements suffer from significant moment-to-moment variability, other molecules found in blood may be able to successfully capture more long-term cortisol exposure. For proof-of-concept, we designed a study to determine if the mean cortisol values collected over a month correlated with *FKBP5* mRNA expression and methylation in blood obtained following 30+ days of saliva collection. We selected these measurements as our initial test biomarkers because dose-dependent changes in gene expression and methylation were observed following exposure to glucocorticoids.(Lee et al., 2011; Yang et al., 2012) In these earlier preclinical studies, we observed that the level of rodent *Fkbp5* gene methylation and expression in blood samples measured following a month of corticosterone exposure reflected the mean glucocorticoid concentration measured during the prior 4-week treatment period. In a human study of Cushing's Disease (tumor-induced hypercortisolism), we showed glucocorticoid-dependent changes in *FKBP5* DNA methylation.(Resmini et al., 2016) Recently, Bali et al. showed that the glucocorticoid prednisone administered to healthy subjects increased *FKBP5* mRNA expression in blood. (Bali et al., 2016) *FKBP5* gene is also notable for its association with neuropsychiatric disorders.(Binder et al., 2004; Klengel et al., 2013; Willour et al., 2009)

This study was conducted as a proof-of-principle to determine if we could identify a blood marker for cortisol load. We hypothesized that *FKBP5* DNA methylation and gene expression derived at the end of a 30-day cortisol collection period would negatively and positively correlate, respectively, with the mean of the cortisol levels collected in the month prior to the molecular assessments. We further hypothesized that self-reported anxiety/depression symptoms and perceived stress levels would be associated with *FKBP5* methylation or gene expression.

2. Methods

2.1. Subjects

We recruited 18–60 year old participants of self-reported European ancestry using local flyers and newspaper advertisements. Three hundred and six individuals completed a brief telephone screen; 113 persons who appeared to be eligible were invited to provide informed consent and participate in a comprehensive assessment at Johns Hopkins School of Medicine. In-person assessments were conducted by a Master's-level interviewer and included contact and demographic information (i.e., date of birth, race/ethnicity, education level, employment status), recent alcohol and drug use assessed using the 90-day Timeline Follow-back (Sobell and Sobell, 1992) and breathalyzer and urine toxicology screens, and past year mental health symptoms obtained using the MINI International Neuropsychiatric Interview for DSM-IV. (Sheehan et al., 1998) Each participant also completed a health checklist that obtained self-reported information on medical history, recent hospitalizations, current prescribed and OTC medications. Persons were excluded from study participation if they reported: using chewing tobacco or a similar product; had a serious medical condition; had taken a medication in the past 3 months that could affect cortisol assay measurements (including antidepressants and anti-anxiety medications); met criteria for a current DSM-5 diagnosis; tested positive for or self-reported recent drug use; exceeded NIAAA guidelines for social drinking; or were currently or had been pregnant or breast feeding within the past 3 months. We also excluded persons who were not going to be continuously available to complete daily study procedures over the 30 days following the assessment. Of the 113 participants who provided informed consent, 24 met exclusion criteria, and 2 were discontinued due to difficulties with the required blood draws. One subject did not complete the daily data collection procedures and dropped out during the study. This resulted in 86 participants who were eligible and provided daily and study visit data. All study procedures were reviewed and approved by the Johns Hopkins School of Medicine Institutional Review Board.

2.2. Procedures

The study protocol consisted of an in-person study visit 1, followed by approximately 30 days of at-home data collection, and then an in-person study visit 2. Eligibility was determined immediately after the assessment interviews and self-report documents were completed at study visit 1. Eligible persons then completed the remainder of the visit 1 procedures, received written instructions and all materials for daily data collection, and had an appointment scheduled for visit 2, which occurred as close to but no less than 30 days following visit 1.

2.3. Psychosocial measurements

At visits 1 and 2, psychosocial measures included the Perceived Stress Scale (PSS),(Cohen et al., 1983) Beck Anxiety Inventory (BAI), (Beck et al., 1988) and the Beck Depression Inventory II (BDI-II).(Beck and Beamesderfer, 1974; Beck et al., 1996) Each day between study visits 1 and 2, participants received telephone, text and/or email prompts for saliva and psychosocial data collection at awakening and at bedtime; subjects were able to select their preferred method and time of reminder contact. Participants were contacted every 15 min for a total of 3 contacts or until the Interactive Voice Response (IVR) system was activated. Subjects also had the opportunity to call into the IVR system and avoid the reminders. During the morning call, participants were instructed to enter their actual awakening and bed times and saliva collection times using the telephone key pad. During the evening call, subjects completed the telephone Stress Assessment Procedure (SAP), which included several items to measure daily stress exposure. Specifically, participants rated their emotional (mental stress, upset, overwhelmed feelings), physical (muscular stress, physical tiredness, fatigue), and overall (combined impact of both emotional and physical stress) stress as well as their ability to manage these stressors. SAP items were rated on an analog scale between 0 (no stress/successfully managed) – 9 (worst ever/unable to manage) using the telephone key pad. Five paper copies of the SAP were supplied to the participant in the event that the telephone system malfunctioned or became unusable for any reason. To promote accuracy and timeliness, data were collected via real-time methods using Survey Monkey and CallFire Interactive Voice Response programming. Participants received a bonus payment if they submitted at least 90% of specimens. At visit 2, participants also completed the health checklist to determine if they had experienced any recent health problems that would confound data collection.