Epigenetic modification of the glucocorticoid receptor gene predicts women's salivary cortisol following a threat to the social self

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INTRODUCTION

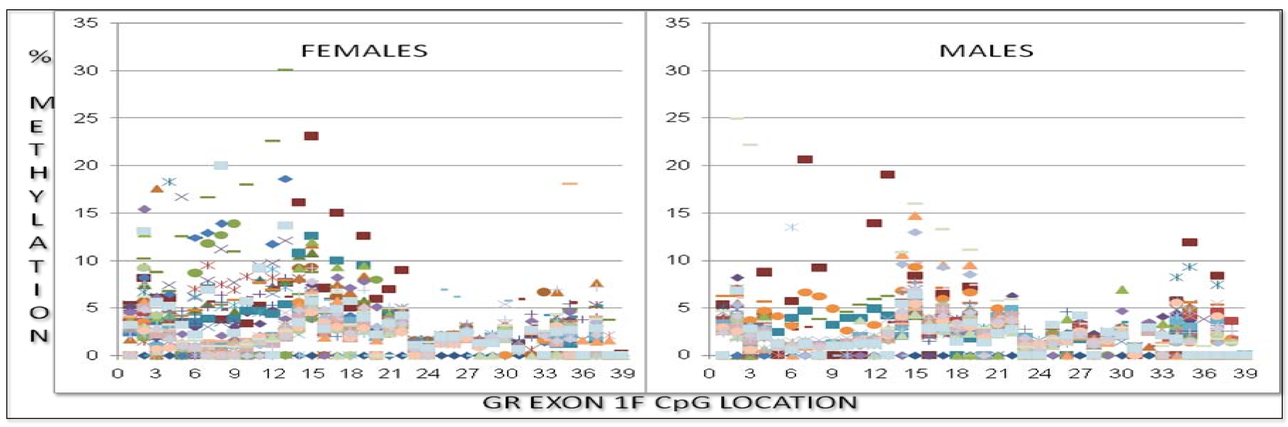
- Evidence suggests that the reactivity of the hypothalamus-pituitary-adrenal axis (HPAA), a major pathway for regulating stress response, is modulated by both genetic and environmental variables.
- A key target of the HPAA release is the glucocorticoid receptor (GR, NR3C1). Methylation of a CpG island in the GR exon 1F promoter is important in regulating gene expression mediated by the nerve growth factor-inducible protein A (NGFI-A) transcription factor.
- Based on animal and human studies, it has been shown that the serotonin transporter (*SLC6A4*) and the estrogen receptor alpha (*ESR1*) modulate HPAA reactivity.
- In the current study we investigated the role of GR methylation levels and the contribution of 5-HTTLPR and ESR1 polymorphisms in explaining gender and individuals differences in cortisol responses to stress.

METHODS

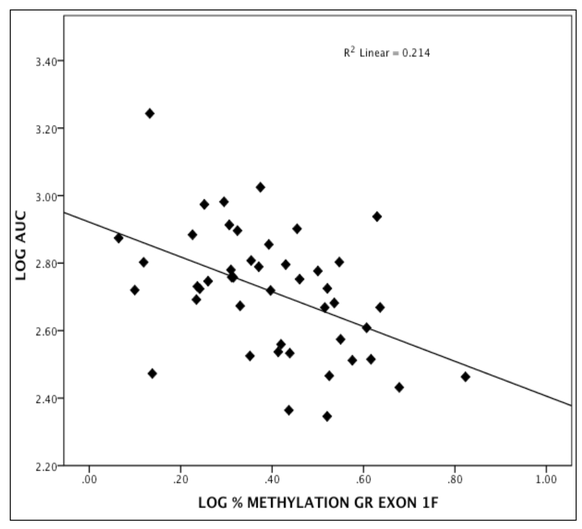
- *Participants* - 92 students from the Hebrew University (46 males, 46 females, average age 25.29, S.D. = 3.6).
- *TSST* - Trier Social Stress Test (TSST) consists of public speaking followed by mental arithmetic task.
  Salivary cortisol was sampled eight times.
- *Bisulfite treatment and methylation analysis* – DNA was extracted from mouthwash samples. Pyrosequencing of bisulfite-treated DNA was carried out by EpigenDx.
- *CpGs selection* - Altogether, we examined the methylation level across 39 CpG sites in GR exon 1F promoter sequence for each subject.
- *Statistical Methods* - All statistical tests were carried out using SPSS version 15. A linear regression model was used to ascertain the effects of sex, GR methylation level and genes on stress response. Statistical normality of the data was checked using the Kolmogorov–Smirnov test.

RESULTS

- Individual differences were observed in methylation levels of the GR exon 1F at individual CpG sites for females and males.



- Overall, women showed significantly greater methylation levels than did men (t=2.538, p=0.013).
- There was a correlation between total cortisol output and average methylation levels at the GR exon 1F in female subjects ($R^2\Delta$=0.214, p=0.001) accounting for 21.4% of the variance.



Linear regression analysis

Dependent variable	AUC		
Regressor	(1)	(2) only Females	(3) only Females
Gender	-0.119** (0.042)		
Average % methylation (log) 39 CpG sites	-0.338* (0.138)	-0.515** (0.149)	-.508*** (0.121)
Serotonin transporter (5-HTTLPR)			0.170** (0.051)
ESR1_dummy1 Estrogen alpha			-0.188** (0.064)
ESR1_dummy2 Estrogen alpha			-0.172** (0.055)
Intercept	2.97*** (0.053)	2.921*** (0.064)	2.936*** (0.075)
Adj. r square	0.153	0.196	0.484
*p<0.05; **p<0.01; ***p<0.001			

- Additionally, variations in the ESR1 and the 5-HTTLPR genes were independent additive predictors of AUC. A significant main effect of 5-HTTLPR ($R^2\Delta$=0.172, p=0.001) and ESR1 ($R^2\Delta$=0.132, p=0.007) was observed on AUC.
- The full model accounted for nearly half of the variance (48%) in total cortisol output.

CONCLUSIONS

- We provide the first evidence that accumulated epigenetic changes at the GR exon 1F correlate with HPAA reactivity.
- Importantly, women show significantly greater methylation across the GR promoter exon 1F compared to men, and the averaged methylation and gene contribution is a highly significant predictor of total cortisol response (AUC) in the TSST.
- This study strengthens the concept that biological stress reactivity mediated by the HPAA, and impacted by both genetic and epigenetic variables, is an important pathway underlying gender differences in stress-related mental health disorders.

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No potential conflict of interest