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# Neuroendocrine stress response is moderated by sex and sex hormone receptor polymorphisms

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ABSTRACT

Sex hormones are significant regulators of stress reactivity, however, little is known about how genetic variation in hormone receptors contributes to this process. Here we report interactions between biological sex and repeat polymorphisms in genes encoding sex hormone receptors, and their effects on salivary cortisol reactivity in a sample of 100 participants (47 men & 53 women; 24.7 ± 3.23 years). Three genes were investigated: estrogen receptors alpha (*ESR1*) and beta (*ESR2*), and the androgen receptor (*AR*). Participants were classified as carrying 'Short' or 'Long' alleles based on median splits of the repeat distribution for each gene. Measures of physiological reactivity were collected before and after exposure to a canonical laboratory stressor and converted to traditional summary measures for analyses. Overall, men exhibited greater cortisol ($p = 0.001$) and mean arterial pressure reactivity ($p = 0.002$), while women displayed elevated heart rate throughout the session ($p = 0.02$). The effect of polymorphisms on salivary cortisol was sex sensitive. *ESR1* was associated with differential reactivity in men ($p = 0.04$), but not women ($p = 0.24$). *ESR2* genotype interacted with sex such that each additional 'Long' allele was associated with a 6.4% decrease in salivary cortisol in men, but a 9.5% increase in the levels of women ($p = 0.02$ for interaction). For the X-linked *AR*, the 'Long' allele was associated with decreased cortisol levels in men ($p = 0.047$), but in women had no effect ($p = 0.75$). Together, these results provide evidence for the saliency of genetic variation in sex hormone receptors on stress reactivity in humans and highlight their important role as mediators of hormonal activity.

## 1. Introduction

Since the original characterization by Walter Cannon and Hans Selye, the dynamics of stress and its relation to health and disease have received growing attention in the literature. The physiological stress response is well conserved across vertebrate taxa, and involves a signaling cascade along the sympathetic-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes of the neuroendocrine system. The HPA signaling cascade results in the release of glucocorticoids (cortisol in humans) from the adrenal cortex into the bloodstream to produce systemic effects throughout the body. Dysregulation of the stress response along this axis has been implicated in an array of diseases including diabetes, depression, cancer, and cardiovascular disease (Cohen et al., 2007). Importantly, individual differences in the timing and magnitude of the stress response contribute to differential incidence and prevalence of stress-associated disorders (McEwen, 2008).

Substantial differences in individual stress physiology can be attributed to genetics. Twin studies have associated up to 62% of the variability in circulating cortisol to genetic background (Bartels et al., 2003). Stress reactivity, the increased cortisol secretion, heart rate, and blood pressure in response to threatening stimuli, is also partially heritable (Federenko et al., 2004), and nearly a dozen different genes have been shown to moderate cortisol responses to acute psychosocial stressors (Foley and Kirschbaum, 2010). Another example of the genomic influence on stress reactivity is sex differences, where the genetic background differs by a full chromosome, i.e. XX vs. XY. Men typically exhibit elevated salivary cortisol secretion during stress as compared to women, whereas in women the magnitude of the salivary cortisol response to stress varies across the menstrual cycle (Kirschbaum et al., 1999). Women in the luteal phase display patterns of salivary cortisol reactivity most similar to those of men, while patterns of women in the follicular phase are significantly lower than men as well as women in the luteal phase (Kirschbaum et al., 1999).

Thus, sex hormones, which deviate in a rhythmic pattern across menstrual cycles, may be important mediators of stress reactivity, at

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least in women. Estrogens have been implicated as a stress buffer in women. For example, estradiol is higher in the follicular phase, when cortisol reactivity is also diminished (Kajantie and Phillips, 2006). Furthermore, the stress reactivity in pregnant women, whose estradiol levels have spiked, is lower than age matched, non-pregnant counterparts, while the stress reactivity in post-menopausal women is higher than that of young-adult women (Kajantie and Phillips, 2006). The role of estrogens in the male stress response is less established and may actually increase reactivity when estrogen is applied transdermally (Kirschbaum et al., 1996).

Instead, testosterone appears to be the primary mediator of the stress response in males. Studies in male rodents have implicated testosterone as an upstream dampener of the HPA axis, inhibiting CRH release by neurons in the hypothalamus (Handa et al., 1994), effectively curbing the stress response before cortisol is secreted and thereby decreasing cortisol release into the periphery. Cortisol and testosterone may have counteracting effects. For example, the link between testosterone and dominance behavior is conditional on low cortisol (Mehta and Josephs, 2010; Popma et al., 2007). Furthermore, circulating testosterone levels decrease following cortisone administration (Cumming et al., 1983). Like estrogen, testosterone may have contrasting effects between sexes. Specifically, testosterone replacement therapy was associated with increased cortisol reactivity in elderly women, but not men (Kudielka et al., 1998).

Importantly, the physiological effects of estrogens and androgens are mediated through cytosolic receptors. Lipid soluble hormones permeate the cell membrane and bind these receptors, which then translocate to the nucleus to modulate gene expression (Beato and Klug, 2000). As a result, differences in sex hormone concentrations between individuals do not necessarily imply differential hormonal activity. Differences in the abundance of receptors and efficiency of hormone-receptor binding also impact function. Notably, DNA sequence variation in polymorphic genes encoding receptors can significantly influence their abundancy and binding affinity (Comings, 1998). Therefore, the lack of consensus regarding the effects of sex steroids could be attributed, in part, to a failure to account for polymorphic genetic variations that modulate receptor activity, availability, and function.

Estradiol binds to two receptors, estrogen receptor alpha (*ESR1*) and estrogen receptor beta (*ESR2*), which are differentially distributed throughout the brain and periphery (Taylor and Al-Azzawi, 2000). Both genes contain repeat polymorphisms thought to play a role in gene expression, although this has yet to be validated; a dinucleotide $(TA)_n$ repeat near the promoter region of *ESR1* (del Senno et al., 1992), and a tandem $(CA)_n$ repeat within the fifth intron of *ESR2* (Tsukamoto et al., 1998). Testosterone acts through the X-linked androgen receptor (*AR*), which, like the estrogen receptors, is non-uniformly distributed throughout the brain and periphery (Sar et al., 1990), with notable differences between men and women (Fernandez-Guasti et al., 2000). One polymorphism of especial interest is a three-nucleotide $(CAG)_n$ repeat within exon one of the gene coding region. This polymorphism changes the receptor structure, and may significantly influence testosterone transactivation (Kazemi-Esfarjani et al., 1995). Specifically, removal of the CAG repeat in both the rat and human *AR* gene resulted in greater transcriptional activity of androgen dependent genes (Chamberlain et al., 1994).

Despite existing literature investigating associations between biological sex, sex hormone concentrations, and stress reactivity, studies investigating how genotypic variability contributes to sex differences in stress reactivity are limited (Pausova et al., 2010). In the current study, we investigated the association between repeat polymorphisms in *ESR1*, *ESR2*, and *AR* and stress reactivity in both men and women. As mediators of the predominate sex hormone in females, we hypothesized that polymorphisms in *ESR1* and *ESR2* would be more strongly associated with differential reactivity in women than men; and accordingly, for *AR* we hypothesized the opposite trend would be observed.

## 2. Methods

### 2.1. Sample recruitment

Young men and women were recruited by word of mouth and on-campus advertising from the college student population at Hebrew University of Jerusalem, Israel. Participants were excluded if they were under the age of 18 or over the age of 35, had a medical history of endocrine or psychiatric illness, smoked, were pregnant in the last year, or were taking any medication (excepting oral contraceptives). In total, 107 individuals participated in the study. Three participants for whom cortisol measurements were not available were excluded from analyses. Another four participants for whom genotypes on neither *ESR1*, *ESR2*, nor *AR* were unavailable were also excluded, bringing the final sample size to 100 (47 men, 53 women). The study was approved by the IRB of Herzog Hospital, Jerusalem and all participants provided written informed consent.

### 2.2. Stressor procedure & psychological indices

Testing was done in a discrete time window, 3:00–6:00 pm, to minimize noise from diurnal changes in cortisol. To further control for variation, participants were asked to refrain from physical activity for at least 2 h before the session, and refrain from brushing their teeth, eating, or drinking anything except water for 1.5 h before the session. Participants feeling ill were rescheduled. The procedure to induce stress was conducted in the Department of Psychology following the Trier Social Stress Test (TSST) paradigm, as previously described (Shalev et al., 2009). Briefly, participants were told they would be interviewing for their dream job while on video, given 5 min to prepare notes, and taken to the testing room. Notes were taken away from participants immediately before the interview began and interviewers were instructed to appear stern and disapproving. Following the five-minute interview, participants completed a serial subtraction cognitive task.

### 2.3. Biological sampling and cortisol assay

Saliva samples for cortisol assays were collected using salivette plugs (Sarstedt, Germany) at eight time points across the 90-minute session: 10 min prior to testing, 1 min prior to testing, immediately after testing, and 10, 20, 30, 45, and 60 min following testing. Saliva samples were stored at room temperature during the session, centrifuged at 4000 rpm at 24 °C for 10 min, and then assayed in an Elecsys 2010 Analyzer using an electrochemiluminescence immunoassay salivary cortisol kit (Roche Diagnostics, USA). The lower detection limit of the assay was 0.5 nM/L. Subject heart rate and blood pressure were collected 10 min prior to testing, 1 min prior to testing, immediately after testing, and 10 min after testing using an automatic blood pressure wrist monitor (Omron R7).

### 2.4. Genotyping

Samples for genotyping were collected prior to stress exposure using two 10 mL sterile tubes containing 10 mL of Aquafresh mouthwash. Both samples were pooled for DNA extraction using the Master Pure kit (Epicentre, Madison, WI). The $(TA)_n$ repeat of *ESR1*, $(CA)_n$ repeat of *ESR2*, and $(CAG)_n$ repeat of the *AR* gene were amplified by polymerase chain reaction (PCR) on a Rotor-Gene 3000 (Corbett life science, Australia). The PCR reaction was conducted in a total volume of 10 mL containing 50 ng DNA (~ 1 mL), 5 mL Thermo-Start Master Mix (Thermo scientific), 2 mL primers (2.5 mM), 1 mL SYTO9 dye, and water to 10 mL. Primers were designed as previously described (Tsezou et al., 2008), and are listed for each gene in Supplementary Table A.1. The forward primer was labeled with 6-FAM and used together with a reverse primer for each gene. The temperature profile was 95 °C for 15 min followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C