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Polymorphisms of genes related to the hypothalamic-pituitary-adrenal axis influence the cortisol awakening response as well as self-perceived stress

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ABSTRACT

The hypothalamus-pituitary-adrenal (HPA) axis is a crucial endocrine system for coping with stress. A reliable and stable marker for the basal state of that system is the cortisol awakening response (CAR). We examined the influence of variants of four relevant candidate genes; the mineralocorticoid receptor gene (*MR*), the glucocorticoid receptor gene (*GR*), the serotonin transporter gene (*5-HTT*) and the gene encoding the brain-derived neurotrophic factor (*BDNF*) on CAR and self-perceived stress in 217 healthy subjects. We found that polymorphisms of *GR* influenced both, the basal state of the HPA axis as well as self-perceived stress. *MR* only associated with self-perceived stress and *5-HTT* only with CAR. *BDNF* did not affected any of the investigated indices. In summary, we suggest that *GR* variants together with the CAR and supplemented with self reports on perceived stress might be useful indicators for the basal HPA axis activity.

1. Introduction

The inter-individual variability in the response to psychological or physical stress represents a central aspect of research in the field of neuroendocrinology. The hypothalamus-pituitary-adrenal (HPA) axis is one of the responsible endocrine systems that maintain physiological homeostasis after challenging situations. After stress, neurons in the hypothalamus release the corticotrophin-releasing hormone (CRH), which subsequently leads to an increased secretion of cortisol from the adrenals. Disturbances in the HPA axis reactivity have been implicated in the pathogeneses of stress-related disorders such as posttraumatic stress disorder (Pacella, Feeny, Zoellner, & Delahanty, 2014) and depression (Plotsky, Owens, & Nemeroff, 1998).

The HPA axis activity could be targeted using different variables: first, detecting the daily profile as reflection of biological body rhythms; second, measuring the stress-induced activation, allowing conclusions on the negative feedback and/or third detecting the cortisol awakening response (CAR) as an indicator for the basal state of the HPA axis. The CAR is the distinct rise in cortisol levels within the first hour of awakening (Pajer, Gardner, Rubin, Perel, & Neal, 2001) and is influenced by psychosocial as well as genetic factors. A meta-analysis revealed associations of job stress and general life stress with increased CAR, and fatigue, burnout as well as exhaustion with reduced CAR (Chida & Steptoe, 2009). Additionally, several studies showed that specific genetic variants are associated with CAR (Derijk, 2009), self-reported chronic stress (Feder, Nestler, & Charney, 2009), and stress-related personality traits (Bouchard & Loehlin, 2001; Bouchard, 2004). The CAR is highly individually stable, with a heritability of approximatly 40% (Wüst, Federenko, Hellhammer, & Kirschbaum, 2000) and can be regarded as a stable biological marker of basal HPA axis activity. Measurable differences in the CAR can be considered as an "endophenotype" as it describes a pattern of the organism's inner process (Gottesman &

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Gould, 2003). The CAR can be measured either as the area under the curve with respect to increase (AUCi) or as the area under the curve with respect to ground (AUCg). AUCi as the primary measure of the CAR represents the timely change of the dynamics of cortisol and is related to the sensitivity of the system (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). AUCg corresponds to the overall level of cortisol released, which is depending on the baseline cortisol level at the time of awakening. AUCi has been considered to be a better indicator of the sensitivity or basal HPA reactivity than AUCg (Pruessner et al., 2003), since the HPA axis (re)activity is the responsiveness of this hormonal system, not a certain hormonal level.

The glucocorticoid (GC) sensitivity represents a key index for individual differences in HPA function (Désautés et al., 2002). Responses of the HPA axis exerted by cortisol are mediated by two hormone receptors, the mineralocorticoid receptor (MR, NR3C2; gene symbols *MR*, *NR3C2*; OMIM *600983) and the glucocorticoid receptor (GR, NR3C1; gene symbols *GR*, *NR3C1*; OMIM *138040). Both nuclear receptors trigger trans-activation and trans-repression of glucocorticoid target genes. The MR has a 10-fold higher binding affinity for GCs and maintains corticosteroid homeostasis by controlling basal HPA axis activity. Furthermore, MR is involved in the appraisal of novel situations and the selection of appropriate coping responses (Derijk, De Kloet, Zitman, & Van Leeuwen, 2011). After substantial increase of GCs, for example after awakening or acute stress (Bamberger, Schulte, & Chrousos, 1996), GR is activated and regulates its target genes. Therewith, GR mediates the regulation of genes involved in the immune response, metabolism and development and plays further an important role in stress related behavior (Derijk, Schaaf, & De Kloet, 2002). Genetic variants have been shown to mediate these processes via direct or indirect modulation of the HPA axis. In table SM-Table 1, an overview is given about the in our study investigated single nucleotide polymorphisms (SNPs) as well as length variations in relevant genes. Previously, two functional *MR* gene variants have been investigated with respect to HPA axis regulation and CAR, the G/C variant located two nucleotides upstream the start codon in exon 1 (rs2070951, *MR*-2G/C) and the G/A variant at codon 180 (rs5522, *MR*I180V). Kuningas et al. reported that carriers of the minor C allele of *MR*-2G/C had lower basal plasma cortisol levels (Kuningas et al., 2007). In addition, the homozygous G allele of *MR*-2G/C was associated with a higher CAR following dexamethasone administration the previous day in men but not in women (Van Leeuwen et al., 2010). After cortisol treatment, the minor G allele of *MR*I180V showed a loss of function *in vitro*. Furthermore, the minor G allele of *MR*I180V was associated with a lower CAR following dexamethasone treatment in men (Van Leeuwen et al., 2010). In the *GR*, several SNPs have been extensively investigated for association with HPA axis function. Here, we investigated the functional important SNPs rs10052950 (*TthIII*I), rs10482605 (NR3C1-1), rs6189 (E22E), rs6190 (R23K), rs41423247 (*Bcl*I), and rs6198 (9beta). The *TthIII*I polymorphism was associated with higher basal cortisol levels in males (Rosmond et al., 2000). The SNPs ER22/23EK, NR3C1-1 and 9beta were associated with relative GC resistance and higher baseline cortisol levels (Kumsta et al., 2007; Manenschijn, Van Den Akker, Lamberts, & Van Rossum, 2009; Van Rossum & Lamberts, 2004). Subjects homozygous for the minor C allele of *Bcl*I had higher *GR* mRNA levels in peripheral blood mononuclear cells (PBMCs) treated *ex vivo* with dexamethasone compared to PBMCs derived from subjects homozygous for the G allele after 24 h (Xiang & Marshall, 2013). In line with this study, *Bcl*I was associated with lower morning cortisol levels *in vivo* after dexamethasone administration (Stevens et al., 2004). The 9beta polymorphism influences the ratio of GRalpha: GRbeta *in vitro* (Derijk et al., 2001). Furthermore, 9beta affects sensitivity to dexamethasone in males (Kumsta et al., 2007).

Besides the glucocorticoid-driven modulation of the HPA axis, serotonergic projections directly facilitate or inhibit HPA axis activity among other factors dependent on their topographically organization (Lowry, 2002). For example in the hypothalamus, serotonin activates corticotrophin-releasing hormone (CRH) neurons as initial impetus of the HPA axis leading to an increased release of CRH (Gold & Chrousos, 2002). Furthermore, the serotonergic system has been identified as being crucial for the prenatal development and early postnatal programming of the HPA axis (Andrews & Matthews, 2004). A key regulator of the serotonin levels within the synaptic cleft is the serotonin transporter protein (5-HTT, SLC6A4; gene symbols *5-HTT*, *SLC6A4*, OMIM *182138), which actively mediates reuptake and recycling of released serotonin following neuronal stimulation. Therefore, the serotonin transporter represents a candidate that plays a role in the early CRH release from pituitary neurons and the subsequent cortisol release (Zhang et al., 2002) and finally determines the basal activity of the HPA axis. Previously, Wüst et al. investigated the impact of the promoter length polymorphism of *5-HTT*, 5-HTTLPR on the CAR and confirmed an influence of that variant on the CAR as endophenotype in a gender-dependent manner (Wüst et al., 2009).

The basal, unstimulated and unstressed HPA axis is further affected via indirect hippocampal input to the hypophysiotrophic neurons regulating the hypothalamic CRH expression. The neuroplasticity of the hippocampus and the development of feedback mechanisms on the HPA axis are modulated via the brain-derived neurotrophic factor (BDNF, gene symbol *BDNF*; OMIM *113505) (Shalev et al., 2009). BDNF is essential for proper development and survival of *e.g.,* serotonergic neurons and is involved in the BDNF dose-dependent decrease of the serotonin uptake (Autry & Monteggia, 2012). Jeanneteau et al. found that BDNF and glucocorticoid signaling regulate hypothalamic CRH expression via an unique mechanism involving cAMP response-element binding protein (CREB) and its coactivator protein, CRTC 2 (Jeanneteau et al., 2012). The most investigated variant of *BDNF*, a SNP (rs6265, Val66Met) in codon 66, has been shown to prevent the activity-dependent release of BDNF (Egan et al., 2003). Furthermore, subjects homozygous for the Met-encoding allele of the Val66Met polymorphism displayed higher ACTH and cortisol levels compared to carriers of the Val allele (Schüle et al., 2006). Therefore, we explored its potential contribution to the CAR and self-perceived stress.

We hypothesize that variants of *GR* and *5-HTT* affect both CAR and self-perceived stress. Whereas, we expect no effect of *MR* on the CAR (AUCi or AUCg) based on previous studies (Van Leeuwen et al., 2010), but we presume *MR* to influence self-perceived stress. Previously, the Trier Inventory for Chronic Stress (TICS) has been used as an index for stress-related HPA axis activity, although without considering genetic influences (Chida & Steptoe, 2009). Furthermore, we assume gender-specific differences in the genetic associations due to previous reports on gender differences in HPA axis response patterns (Kajantie & Phillips, 2006; Uhart, Chong, Oswald, Lin, & Wand, 2006). In general, with regard to the scrutinized genetic influences on HPA axis activity, which is reflected by the sensitivity of this hormonal system, we expect to find influences of genotypes with regard to the AUCi rather than the alternative AUCg.

2. Methods

2.1. Participants

In total, the sample consisted of 231 participants from the University of Trier. Of them, 217 healthy men and women (115 females, mean age years = 23 ± 2.8; BMI 22.4 ± 0.25) completed the experimental procedures. Ethnic data were not collected, as population stratification was unlikely in our sample. Recently, Steffens et al.