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# Genetic variation of the mineralocorticoid receptor gene (*MR, NR3C2*) is associated with a conceptual endophenotype of "*CRF-hypoactivity*"

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ABSTRACT

Recently, the „conceptual endophenotype" approach has been proposed as a means to identify subgroups of patients affected by stress-related psychiatric disorders. Conceptual endophenotypes consist of patterns of psychological, biological, and symptomatic elements. We studied a sample of patients seeking help for psychosomatic and stress-related disorders (total N = 469), who were evaluated with a diagnostic instrument that integrates psychological and biological data to derive 13 endophenotypes, or Neuropattern. The goal of this study was to explore associations between common variations of the mineralocorticoid receptor gene (*MR, NR3C2*), and the 13 conceptual endophenotypes of Neuropattern, as well as with the respective biological and symptom measures. A common haplotype of the MR, comprised of two functional single nucleotide polymorphism (rs2070951 G/C & rs5522 A/G), was associated with the conceptual endophenotype *CRF-hypoactivity,* characterized by low cortisol levels at awakening and a symptom constellation often observed in atypical depression. Homozygous carriers of the G-A haplotype (haplotype 1), previously associated with reduced dispositional optimism, increased levels of rumination and higher risk for depression, more frequently endorsed this Neuropattern. In addition to the overall association between *MR* variation and *CRF hypoactivity,* we observed in the whole sample significant associations between *MR* haplotypes and cortisol awakening response patterns, as well as with symptoms that characterize the *CRF hypoactivity* endophenotype. If replicated, *MR* haplotype 1 might serve as a vulnerability marker for a disorder class characterized in biological terms by reduced cortisol levels, and in terms of symptom constellation by features often observed in atypical depression.

## 1. Introduction

One of the long-term goals of psychiatric research is to realize personalized treatment approaches based on the understanding of disease processes. The US National Research Council (2011) advocates a multi-level approach, integrating molecular, clinical, and environmental data, and health outcomes in a dynamic, iterative fashion. The resulting knowledge network generates subtypes mainly from individual characteristics of the genome, epigenome, microbiome, and exposome and the patient´s signs and symptoms. Validated subtypes are expected to allow personalized diagnostic and therapeutic treatments.

Comparable to the NRC approach, a new translational strategy based on conceptual endophenotypes was introduced by Hellhammer et al. (2012). The authors defined contextual and formal criteria of conceptual endophenotypes, outlined criteria for filtering and selecting information, and described how conceptual endophenotypes can be validated and implemented at the bedside (Hellhammer et al., 2018). Conceptual endophenotypes consist of patterns of psychological, biological, and symptomatic elements and are iteratively developed and validated, linking research and clinical evidence. Conceptual endophenotypes attempt to engineer a bridge between the bench and the bedside and may offer a new way to improve the prediction, preemption and treatment of stress-related disorders.

Additionally, conceptual endophenotypes may serve as a tool to explore and detect the clinical relevance of gene variants. We here report an attempt to explore associations between haplotypes of the

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**Box 1**
Conceptual Endophenotypes or Neuropattern

The diagnostic tool comprises 13 distinct conceptual endophenotypes, or Neuropattern, which reflect different types of stress system dysregulations and can be grouped as described below. Each Neuropattern is conceptualized by a specific pattern of psychological, biological, and symptom measures (Hellhammer and Hellhammer, 2008; Hellhammer et al., 2012, 2018). *CRF hypoactivity*, for instance, is characterized by a low cortisol level at awakening, and symptoms of hypoarousal, hypersomnia, significant weight gain or increase in appetite, pronounced fatigue, loss of energy, and lethargy (also typically observed in atypical depression).

mineralocorticoid receptor gene (*MR, NR3C2*) and conceptual endophenotypes of the stress response network. We applied a conceptual endophenotype-based tool (Neuropattern), which focuses on dysregulations of noradrenergic, serotonergic, sympathetic and parasympathetic systems, as well as of the hypothalamic-pituitary-adrenal axis (HPAA; Hellhammer and Hellhammer, 2008; Hellhammer et al., 2018). The Neuropattern approach is based on 13 conceptual endophenotypes; each of which is defined by a characteristic pattern of psychological, biological, and symptom variables (Box 1).

Following a candidate gene approach, we tested whether variation of a gene coding for a key regulator of the HPA axis, namely the mineralocorticoid receptor (*MR, NR3C2*), might be associated with Neuropattern endophenotypes. The MR was chosen because of in-depth characterization of common MR haplotypes at the molecular, cellular, systems and behavioral level (de Kloet et al., 2016; DeRijk et al., 2011), which has provided converging evidence for their physiological and clinical relevance, and suggested that MR gene haplotypes might serve as valuable biomarkers for a subgrouping of stress-related disorders.

| Concept | Dsyregulation | Neuropattern |
|---|---|---|
| **Ergotropy**<br>*Ergotropy refers to catecholaminergic/ sympathetic functions associated with arousal, mental or physical work, and alertness* | *hyperreactivity, hyper- and hypoactivity of norepinephrine neurons originating from the locus caeruleus, sympathetic hyperactivity and hyperreactivity* | • NA hyperreactivity,<br>• NA hypoactivity<br>• NA hyperactivity<br>• Sympathetic hyperactivity<br>• Sympathetic hyperreactivity |
| **Trophotropy**<br>*Trophotropy refers primarily to central and parasympathetic functions that underlie regeneration, recovery, and protection against stress overload* | *hyperreactivity and hypoactivity of serotonergic neurons from the dorsal and medial raphe nucleus* | • Serotonin hypoactivity<br>• Serotonin hyperreactivity |
| **Glandotropy**<br>*Glandotropy refers to the activity of the different central and peripheral components of the HPA axis that are associated with mobilization of energy, prevention of an disinhibited stress response, and psychological states such as anticipation, worry, lack of control, and ego involvement* | *hyperactivity, hyperreactivity and hypoactivity of hypothalamic CRF and CRF/AVP neurons, elevated and diminished cortisol release from the adrenals, and glucocorticoid receptor resistance* | • CRF hypoactivity<br>• CRF hyperreactivity<br>• GR resistance<br>• Cortisol hypoactivity<br>• Cortisol hyperactivity<br>• CRF hyperactivity |

The most extensively studied MR haplotype is based on two common SNPs located in the promoter region (rs2070951) and in the first coding exon (exon 2; rs5522) of the MR gene. Three common haplotypes (haplotype 1 (GA), frequency 48.8%; haplotype 2 (CA), frequency 41.9%; haplotype 3 (CG), frequency 9.3%) and one very rare (haplotype 4 (GG), frequency less than 0.1%) haplotype have been observed (de Kloet et al., 2016; van Leeuwen et al., 2010). Functional characterization of the haplotypes showed higher transactivation potency and a higher expression of MR driven by haplotype 2 (van Leeuwen et al., 2011). With regard to HPA axis regulation, associations between these haplotypes and the cortisol awakening response as well as with the ACTH, cortisol and heart rate responses to psychosocial stress exposure have been reported. A sex-specific association between MR haplotypes and the cortisol awakening response following administration of 0.25 mg dexamethasone was found (van Leeuwen et al., 2010): Male homozygous carriers of haplotype 1 showed the largest post dexamethasone cortisol increases, whereas there were no differences in women, and no differences were observed in the awakening cortisol responses without prior dexamethasone treatment. Klok et al. (2011) did also not observe an effect on the CAR in medication-free participants, however, the rs2070951 GG genotype was associated with larger cortisol levels and increases in individuals using SSRIs. In a sample of school teachers, stress reactivity and chronic stress levels were investigated. Individuals homozygous for haplotype 2 displayed the largest neuroendocrine and heart rate responses following the TSST. The same haplotype was associated with lower scores on the TICS subscales *excessive demands at work* and *social overload* (van Leeuwen et al., 2011), higher dispositional optimism, implicit happiness and less rumination as well as reduced thoughts of hopelessness (Hamstra et al., 2017, 2015; Klok et al., 2011). Lastly, it was shown that haplotype 2 is associated with a lower risk of depression in females (Klok et al., 2011), especially in combination with trauma (Vinkers et al., 2015). However, haplotype 1 and 3 were an advantage for males when sex and early life adversity were taken into account (Vinkers et al., 2015) (Box 2).

In the current study, we explored associations between MR gene haplotypes and the 13 conceptual endophenotypes of Neuropattern, as well as the respective biological, psychological, and symptom measures.

## 2. Methods

### 2.1. Sample description

Study population consisted of 469 subjects (288 females and 181 males) deriving from an in- and outpatient population. Inpatients (n = 92) were recruited from a department of behavioral medicine and psychosomatics (Rehabilitation Center Seehof, Teltow, Germany). Outpatients (n = 377) were recruited in collaboration with general practitioners regarding stress related disorders in Rhineland-Palatinate.

**Box 2**
Brain mineralocorticoid receptors

The nuclear receptor subfamily 3 group C member 2 (*NR3C2*) gene encodes the mineralocorticoid receptor (MR) which occurs as aldosterone-selective in n. tractus solitarii and circumventricular organs involved in salt appetite and as cortisol/corticosterone-preferring in limbic forebrain structures. Aldosterone selectivity is because of cellular colocalization of the MR with 11β-hydroxysteroid dehydrogenase which breaks down cortisol to inactive cortisone intracellularly.

MR binds cortisol and corticosterone with a tenfold higher affinity than to NR3C1, the glucocorticoid receptor (NR3C1, GR), which are both nuclear receptors that regulate gene transcription. Because of its high binding affinity, MR is largely occupied even under basal conditions, while GR becomes gradually activated during the ultradian / circadian rise and after stress Circadian activities, stress coping and adaptation are coordinated in a complementary manner by MR and GR (de Kloet et al., 2005).

Brain MR maintains a high excitability of limbic neurons relevant for the tone of excitatory outflow of limbic afferents towards autonomic, neuroendocrine and behavioral substrates of valence assessment, decision-making, emotional expression and cognitive performance. In addition to this MR-dependent genomic control of neuronal networks, brain MR also mediates rapid non-genomic actions that promote glutamate release and rapidly increase excitability during salient events (Joels et al., 2012). Rising cortisol levels after stress progressively shift energy allocation from a MR-regulated salience network to (non)genomic GR-dependent executive functions (Hermans et al., 2014; Vogel et al., 2016). The latter can be divided in three categories (i) membrane receptors involving endocannabinoid release (ii) transrepression that curtails stress-induced excitability and signaling cascades to prevent them from overshooting and becoming damaging (iii) transactivation by interaction of MR-GR heterodimers and, with higher cortisol concentrations, of GR homodimers with glucocorticoid response elements ((GREs) to secure energy resources and promote memory storage for future use (de Kloet et al., 2018; Hill and Tasker, 2012; Reichardt and Schutz, 1998).

Recent studies have demonstrated that the (non)genomic limbic MR-mediated actions regulate selective attention, appraisal of sensory information, decision-making and selection of coping styles. In addition, blockade of MRs interferes with learning and retrieval of stressful information (de Kloet et al., 2018; Joels et al., 2012). Clinical studies using pharmacological manipulation of MR as well as genetic association studies have demonstrated a role of MR in aspects of emotion regulation and cognitive performance in healthy and depressed individuals (de Kloet et al., 2016).

Subjects were aged between 17 and 77 years with a mean age of 44.59 years (SD 10.49). Clinical diagnoses were primarily depression, anxiety disorders and somatoform disorders. Exclusion criteria were severe psychotic disorders, pregnancy and current breastfeeding. The protocol of the inpatient study was reviewed and approved by the institutional review board of the Deutsche Rentenversicherung Bund (Federal German Pension Insurance Agency). The protocol of the outpatient study was approved by the ethical commission of the medical association of the state Rhineland-Palatinate (Ref. No: 837.296.09 (6802) an registered by the U.S. National Institutes of Health (ClinicalTrials.gov-Identifier NCT01062880). Both studies were conducted in accordance with the declaration of Helsinki.

### 2.2. Neuropattern™ questionnaires

The Neuropattern™ questionnaires (NPQ) were designed especially for Neuropattern™ and are part of the Neuropattern™ kit (Hellhammer and Hellhammer, 2008; Hellhammer et al., 2012). The kit contains the following questionnaires: NPQ-A, NPQ-P, NPQ-PSQ, NPQ-S. The NPQ-A is an anamnestic questionnaire to be completed by a physician, recording medical history, treatments and medical data (blood pressure, heart rated, body-mass index etc.). The NPQ-P asks for specific characteristics of physical and psychosomatic stress symptoms assigned to the status of the single Neuropattern™ interfaces. The NPQ-PSQ assesses pre-, peri- and postnatal stress with the help of close relatives and birth records. The NPQ-S acquires the subjective quality of the stress reaction and different aspects of exhaustion with reference to the last four weeks, as well as impairments by major stressful life events (Boyle and Hellhammer, 2013; Hellhammer and Hellhammer, 2008; Hellhammer et al., 2012; Waeldin et al., 2015, 2016). All NPQs, except the NPQ-A are filled in by the subjects themselves.

With the PHQ-D, the German version of PRIME MD Patient Health Questionnaire (Spitzer et al., 1999) was used, which enables a valid diagnostic of mental disorders, as well as severity of symptoms in a timely manner (Gräfe et al., 2004; Löwe et al., 2002).

### 2.3. Cortisol awakening response

In addition to the Neuropattern™ questionnaires on three consecutive days 16 saliva samples for the assessment of the cortisol awakening response (CAR) and the diurnal cortisol rhythm were collected.

The CAR was assessed on two consecutive days, with saliva samples collected at awakening, and 30, 45, and 60 min thereafter with Salivette devices (Sarstedt, Nuembrecht, Germany). Two additional samples were taken at 1500 and 2000 h. On the evening of the second day, subjects were given 0.25 mg of dexamethasone orally, to assess feedback sensitivity of the HPA axis. Here, only CAR results are reported. Participants reveived the Neuropattern Testset, as illustrated in Boyle and Hellhammer (2013, p.243). The testset comes with detailed instructions how to use the salivettes, and how to handle food intake and smoking, brushing teeth, etc. Directly after awakening and to the subsequent time points, patients removed the specific salivette from its tagged position in the testset, and put it back afterwards. While in the inpatient sample, the study nurse controlled the procedure at awakening, the outpatients were advised to set an alarm clock 15 min before habitual awakening time and start with the first sample immediately. Salivettes were stored at -20 °Celsius until further analyses. Free cortisol levels were assessed via a competitive immunoassay (Dressendo¨rfer et al., 1992) at the laboratory of the Department of Biological and Clinical Psychology, University of Trier. Intra- and inter-assay variance of around 5%. Means of the two diurnal profiles were used. If an awakening response could only be identified for one day (Cortisol at awakening > Cortisol at 30 min), the CAR of the other day was excluded from analyses.

### 2.4. Integration of diagnostic information

After data collection, diagnostic information was sent to the Neuropattern™ laboratory, where psychological, biological and symptom data were analysed and underwent an integrated pattern evaluation. The software-aided data analysis followed a priori defined criteria for the single Neuropatterns™ (Hellhammer and Hellhammer,

2008; Hero et al., 2012). CRF-hypoactivity was conceptualized by information commons on psychological, biological and symptom measures, which are considered to be associated with low activity of CRF-neurons in the central nervous system. A defined number of variables has to be fulfilled for each of the following three categories: symptoms (depression, hypersomnia, fatigue, muscle weakness, weight gain, etc), psychological variables (lethargy, inactivity, increased appetite, etc), and biological measures (low cortisol levels at awakening and a super-suppression of cortisol levels to 0.25 mg dexamethasone (Fries, 2008)).

### 2.5. Statistical analyses

Chi-squared tests were used to test the association between MR haplotypes and the 13 Neuropattern. Chi-square tests were also used to test the association between MR haplotypes and symptom measures. General Linear Models (GLMs) were computed to assess the repeated measures effect time, the between-subjects effect genotype as well as the interaction time × genotype for the cortisol awakening response. Because of previous reports of sex specific effects of MR haplotypes on cortisol levels, sex was included as an additional between subject factor in a subsequent analysis.

### 2.6. Genotyping for MR haplotypes

DNA was collected via saliva samples using the Oragene® OG-500 collection kit (DNA Genotek, Kanata, Canada). DNA was extracted using an adapted protocol of the Gentra Puregene DNA purification kit (Qiagen, Hilden, Germany) at the Max-Planck Institute of Psychiatry, Munich. SNPs were analysed on a Sequenom platform using the iPlex technology (Sequenom, San Diego, USA) in a multiplex assay.

Genotyping was successful for 451 out of 469 patients. For the rs2070951, 23.4% were homozygous for the G allele, 47.2% were heterozygous, and 24.4% were homozygous for the C allele. For rs5522, the following genotype frequencies were observed: 74.9% AA, 19.4% GA, and 2.1% GG. Observed allele frequencies corresponded to those previously reported (de Kloet et al., 2016), and there were no deviations from Hardy Weinberg equilibrium ($X^2 < 1.9$; $p > .17$).

As rs5522 and rs2070951 are in linkage disequilibrium (de Kloet et al., 2016; van Leeuwen et al., 2011), we followed a haplotype based approach. Haploview (Barrett et al., 2005) was used to calculate linkage disequilibrium among the two *MR* SNPs. The estimated linkage between rs2070951 and rs5522 was D' = 1 (confidence bounds 0.91–1) and $r^2 = 0.137$. Haplotypes were estimated and assigned to each individual using UNPHASED (Dudbridge, 2008). As in previous studies, three haplotypes were detected (haplotypes and frequencies are shown in Fig. 1). In all analyses, we compared homozygous carriers of haplotype 1 (2 copies), to carriers heterozygous for haplotype 1 (one haplotype 1 and either one haplotype 2 or 3 copy), to homozygous non-carriers of the GA diplotype (0 haplotype copies).

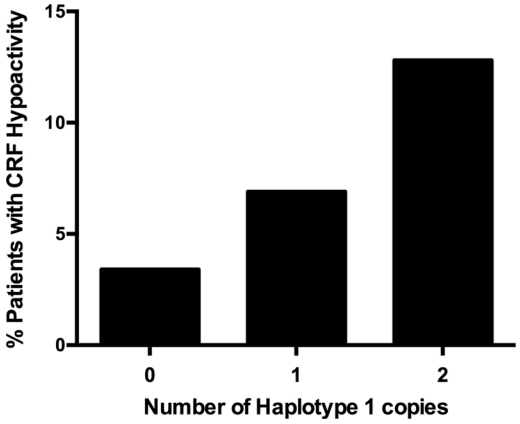


**Fig. 2.** Association between MR haplotypes and CRF hypoactivity pattern. 12.8% of patients carrying two GA allele copies qualified for the pattern, whereas 6.9% and 3.4% of heterozygous and non-carriers qualified, respectively.

## 3. Results

### 3.1. Associations between MR gene haplotypes and conceptual endophenotypes

In a fist step, we explored associations between MR haplotypes and the 13 conceptual endophenotypes of Neuropattern. In the outpatient sample, only the pattern *CRF hypoactivity* was significantly associated with MR gene haplotypes ($X^2_{(2)} = 6.02$, $p = .049$). Individuals homozygous for haplotype 1 were more likely to qualify for the *CRF hypoactivity* pattern (Fig. 2). In the inpatient sample, no association between MR haplotypes and conceptual endophenotypes were observed ($X^2_{(2)} = 0.93$, $p = .629$).

### 3.2. Associations between MR gene haplotypes and biological, and symptom measures of CRF-hypoactivity

In a second step, given the overall association between MR haplotypes and the *CRF hypoactivity* construct, we further explored the association between genotype and biological and symptom measures of CRF hypoactivity in the whole sample. Criteria that characterize the *CRF hypoactivity* construct resemble clinical features of atypical depression, with symptoms such as hypersomnia, fatigue, hyperphagia and weight gain. In addition, low cortisol levels at awakening are considered as characteristics for *CRF hypoactivity*. Results show that individuals homozygous for haplotype 1 had a significantly lower cortisol awakening response. Patients with 0 or 1 copy showed virtually the same cortisol levels at awakening and at 30, 45 and 60 min thereafter, whereas carriers of two haplotype 1 copies showed lower cortisol levels across all time points (main effect haplotype: $F_{2,432} = 3.36$, $p = 0.036$; Fig. 3). There were no *time* by *sex* or by *haplotype* by *sex* effects (all $p > .12$).

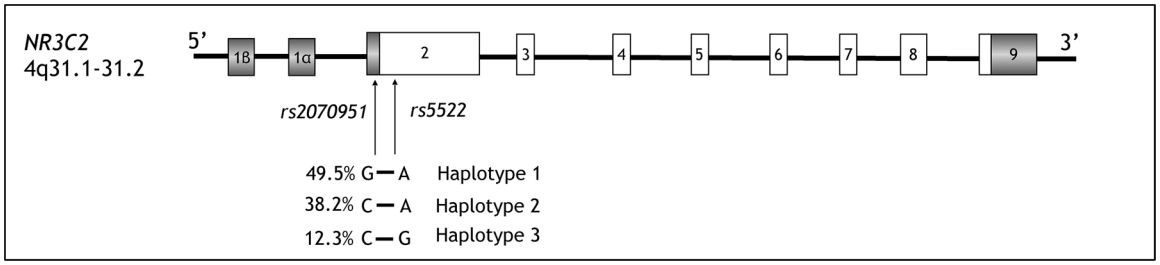


**Fig. 1.** Organization of the human mineralocorticoid receptor gene (*MR; NR3C2*) and the location of the two assayed SNPs rs2070951 and rs5522. Indicated haplotype frequencies are those observed in our sample.

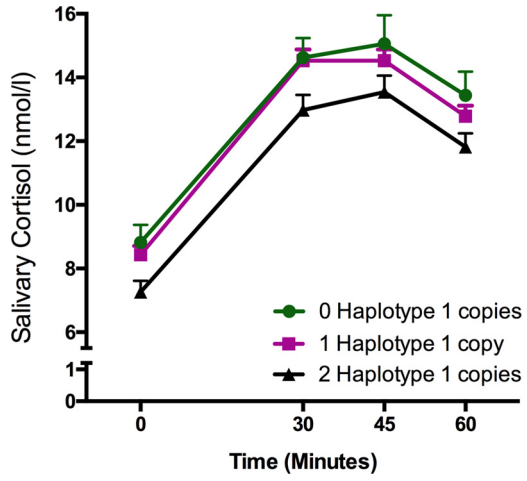


**Fig. 3.** The cortisol awakening response in carriers of 0,1 and 2 copies of the GA allele in the whole sample (n = 451).

**Table 1**
Symptoms characterizing the CRF hypoactivity construct and the association with MR haplotypes.

| Symptoms characterizing the Neuropattern *CRF hypoactivity* | P value |
|---|---|
| Depressivity | 0.013 |
| Hypersomnia | 0.037 |
| Muscle weakness | 0.021 |
| Feeling as being paralyzed | 0.032 |
| Lethargy | 0.007 |
| Adynamia | 0.003 |
| Inactivity | ns |
| Fatigue | ns |
| Exhaustion | 0.011 |
| Increased appetite | ns |
| Weight gain | 0.031 |
| Psychological symptoms of atypical depression | ns |

On the level of symptom measures, we observed significant associations between MR haplotypes and most symptoms (yes/no) of the conceptual endophenotype *CRF hypoactivity* (see Table 1), with carriers of 2 haplotype 1 copies endorsing symptoms more frequently.

## 4. Discussion

Recently, the „conceptual endophenotype“ approach has been proposed as a means to identify subgroups of patients affected by stress-related psychiatric disorders based on biological, psychological and symptom measures. The assessed biomarkers comprise peripheral stress measures, but currently genetic information are not yet routinely assessed. Here, we found evidence for an association between common variation of the mineralocorticoid receptor gene, a key player in the regulation of the HPA axis, and one specific conceptual endophenotype (or Neuropattern) termed *CRF hypoactivity,* which shares many features with atypical depression.

In psychiatry research, there is an on-going discussion about the validity of categorical psychiatric diagnoses, based on symptoms, course, and in part also disability. It has been argued by many that such categorical approaches fail to represent the complex nature of psychiatric disorders and do not capture fundamental underlying mechanisms of dysfunction (Fava et al., 2018; Insel and Cuthbert, 2015; Linden and Rath, 2014; Nagar et al., 2018). Several strategies have been put forward to establish a scientific base for a reorganization of diagnostic system (Alhajji and Nemeroff, 2015; Prendes-Alvarez and Nemeroff, 2018; Turetsky et al., 2007; Yu et al., 2016), with the long-term goal of introducing concepts of cause and mechanism (McHugh, 2009). These strategies, including the Research Domain Criteria (RDoC) initiative (Kozak and Cuthbert, 2016), have in common their attempt to integrate multiple levels of information from biological (e.g. genetics, epigenetics, neuroimaging) and non-biological data (e.g. family and personal history).

Guided by the NRC approach on Precision Medicine, a new translational strategy was recently introduced. The strategy based on „conceptual endophenotypes“ has in common with the other strategies that data across various different layers of analysis are integrated to derive clusters of endophenotypes to discriminate patient sub-groups. In contrast to the other approaches - predominantly driven by a diverse set of concepts and hypotheses that are not grounded in an overarching theoretical model - the "conceptual endophenotype“ approach focusses on psychobiological stress systems and selected well-defined biological structures and mechanisms that are closely implicated in stress-related disorders. The difference to other approaches using unsupervised approaches to define new clusters as performed e.g. for schizophrenia (Wessman et al., 2009), ADHD (Karalunas et al., 2014), or depression (Drysdale et al., 2017), is that the conceptual endophenotype approach is guided by a-priori knowledge about function of stress physiology systems. The focus was thus solely on measures of the interface in the crosstalk between the brain and peripheral systems under stressful conditions, e.g. the HPA axis, autonomic nervous system, and selected components of the central nervous system, namely the locus caeruleus–noradrenergic (NE) and the dorsal raphe–serotonergic (5-hydroxytryptaminergic, 5-HT) systems. In order to derive subgroups of patients affected by stress-related disorders, characteristic pattern of psychological, biological, and symptom variables were defined, resulting in 13 conceptual endophenotypes, or Neuropattern.

Currently, the Neuropattern approach does not incorporate markers commonly used by other approaches, such as immune system markers, epigenetic modifications, or genetic variants. However, epi-/genetic markers might in the long run help to refine functional understanding of the dysregulation in the proposed stress circuits and facilitate basic research on the genetic and epigenetic determinants of these endophenotypes.

In line with the conceptual endophenotype approach with its focus on stress physiology systems, and in order to enhance chances of finding an association, we aimed to select a gene with key regulatory function for the HPA axis, and we furthermore aimed to focus on genetic variants with known functionality and replicated associations with stress system function. MR haplotypes have been thoroughly characterized at the molecular, cellular, systems and behavioral level (de Kloet et al., 2016). Hence we explored associations between these haplotypes and the 13 conceptual endophenotypes of Neuropattern, Our study showed that only one of these endophenotypes, *CRF-hypoactivity*, was associated with MR gene variation. A common haplotype, conferring reduced MR expression and transactivation in vitro, and previously associated with more chronic stress experience, reduced dispositional optimism, increased levels of rumination and higher risk for depression, was associated with a reduced cortisol awakening response and several psychological symptoms characteristic for the conceptual endophenotype *CRF-hypoactivity*. Criteria that characterize this construct clinically resemble features which are observed in atypical depression, a depression subtype listed as ICD-10 F32.8., which presents in many ways an antithesis of the melancholic type. The increased food intake, excessive sleepiness, fatigue, etc in atypical depression had led to the suggestion of CRF hypoactivity as an important trigger of atypical depression (Fries, 2008; Gold, 2015; Gold and Chrousos, 2002, 2013).

The prevailing pathophysiological model of the etiology of psychiatric disorders is that of an interaction between genetic vulnerability and environmental risk factors. Exposure to stress is a significant and causal risk factor for mental disorders (Kendler et al., 1999), and dysregulations of biobehavioral stress systems are thought to be mechanistically involved (Meaney et al., 2007). It is unclear, however, whether

there are specific genetic markers or a specific constellation of genetic variants are related to specific alterations in the function of stress response systems. Conceptual endophenotypes might guide the identification of such biomarker once the sample sizes needed for genome-wide studies are available. Our preliminary results suggest that the investigated MR haplotype 1 might be a vulnerability marker for a disorder class characterized in biological terms by hypoactivity of CRF signaling, and in terms of symptom constellation by features often observed in atypical depression.

We can only speculate about potential mechanisms that underlie the association between functional MR variation and differences in the CAR specifically, and HPA axis regulation in general. *in vitro* data show that haplotype 2 increases MR expression. Conversely, haplotype 1 - associated with a lower CAR in the present study - leads to a relative decreased MR expression. A number of animal studies have shown that higher MR expression in the brain leads to a more dynamic HPA axis response, and is associated with less anxiety-like behaviour (Brinks et al., 2009; Rozeboom et al., 2007; van Eekelen et al., 1992). Combining the findings of increased HPA axis dynamics to stress with those of a more favorable behavioral profile in haplotype 2 carriers (Hamstra et al., 2017, 2015; Klok et al., 2011) support the notion of better stress handling in haplotype 2 carriers, and further support low-expression haplotype 1 as a vulnerability genotype - as reported in the present study. However, it is not straightforward to explain *in vivo* results based on the *in vitro* data, and the precise mechanism how the putative increased MR expression leads to effects on HPA axis regulation and variation in stress-related behaviors is still unknown. For instance, in pharmacological animal and human studies MR antagonists icv invariably produce elevated basal and stress-induced ACTH and corticosterone release (Dodt et al., 1993; Ratka et al., 1988). In mouse lines genetically selected for coping style, in the dominant animal the higher hippocampal MR expression correlates with reduced corticosterone secretion (Veenema et al., 2003). This finding corroborates with the reduced HPA axis activity in mutant mice genetically engineered to express excess MR over GR (Harris et al., 2013).

Following limitations need mention. First, there was no *a priori* expectation of an association between MR gene variation and a particular conceptual endophenotype, and the observed results would not survive correction for multiple testing (13 conceptual endophenotypes). This study is exploratory in nature and results need to be replicated before firm conclusions can be drawn. Second, it remains unclear whether the association between MR haplotype and the conceptual endophenotype CRF hypoactivity can be functionally explained through direct effects on CRF availability as no direct measure of CRF was available. Further, it is of note that we did not observe a sex-specific effect of MR gene variation on cortisol levels, in contrast to other reports (Klok et al., 2011; van Leeuwen et al., 2010; Vinkers et al., 2015).

In conclusion, we studied a sample of patients seeking help for depression, anxiety disorders and somatoform disorders who were diagnosed with the Neuropattern diagnostic instrument, a tool to derive conceptual endophenotypes for subgrouping patients with stress-related disorders. An association was observed between a well-characterized and functional genetic variant of the MR gene and the conceptual endophenotype CRF hypoacitivity. If replicated, MR gene variation might serve as a biomarker for a syndrome characterized by HPA axis hypoactivity and symptoms of atypical depression.

## Conflicts of interest

RdR is Chief Scientific Officer of DynaCorts and RdK is Scientific Advisor of DynaCorts. All other authors reported no biomedical financial interests or potential conflicts of interest.

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