Mineralocorticoid receptor gene variants : implications for stress, blood pressure and personality

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6

Two haplotype blocks in the Mineralocorticoid Receptor (MR) associate with neuroticism but not with mood and anxiety disorders

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Summary

Stress causes activation of the hypothalamic-pituitary-adrenal (HPA)-axis, resulting in the secretion of cortisol, which feeds back on stress-induced HPA activation and facilitates behavioral adaptation. These effects exerted by cortisol are mediated by two brain corticoid receptor populations, i.e. the mineralocorticoid receptors (MR) and the glucocorticoid receptor s(GR), MR and GR operate in complementary fashion in control of the HPA axis and behavior. MR is mainly expressed in limbic brain structures regulating predominantly the initial reaction to psychosocial stressors, which is subsequently suppressed by the ubiquitous present GR.

Dysregulation of HPA-axis reactivity has frequently been reported in patients with mental disorders. In addition, the personality trait "neuroticism" is regarded as a vulnerability factor for depression. Twin studies demonstrated that cortisol levels, psychopathology and neuroticism are heritable. Previously, it was demonstrated that genetic variation in the MR is functional *in vitro* and influences responses to a psychosocial stressor.

In the current study, involvement of the MR in neuroticism and psychopathology was tested by measuring common genetic polymorphisms in the MR-gene in a cohort of healthy controls (n=50) and patients with mood and/or anxiety disorders (n=100). In this relatively small cohort there was no difference in MR genotype distribution between patients and controls. However, the genetic variation in the MR appeared to be associated with neuroticism in patients (p=0.02).

In conclusion, these preliminary findings suggest that genetic variation in the MR associates with neuroticism, indicating involvement of the MR in human behavior. This finding is in support of previous evidence from animal studies linking variation in MR function with emotional and cognitive aspects of the stress response.

Introduction

Stress causes activation of the hypothalamic-pituitary-adrenal (HPA)-axis, resulting in the secretion of cortisol, which facilitates behavioral adaptation. Dysregulation of the HPA-axis has frequently been reported in patients with mental disorders. Patients with severe depression often have elevated cortisol levels. Furthermore, the personality trait "neuroticism" that is characterized by chronic negative affect is regarded as a vulnerability factor for depression (Weinstock and Whisman, 2006; Ormel et al., 2004; Clark et al., 1994).

Although the magnitude of heritability differs largely between studies, it is clear that cortisol levels, mood and anxiety disorders and neuroticism are heritable (reviewed by (Flint, 2004). In addition several studies showed that cortisol levels are positively correlated with neuroticism. Therefore genetic variability in factors regulating the stress system might underlie differences in neuroticism and susceptibility to psychopathology.

Central in the regulation of the HPA-axis by cortisol are two brain corticoid receptor population, the high affinity mineralocorticoid receptor (MR) and the low affinity glucocorticoid receptor (GR). GR is expressed throughout the whole body and mediates via the elevated cortisol levels the metabolic, immunological, cardiovascular and behavioural adaptations in response to stress. MR binds in the limbic brain not only cortisol but also aldosterone with high affinity, but since the concentration of cortisol is much higher the brain predominantly *sees* the latter steroid. In aldosterone target tissues such as sweat glands, distal colon, kidney and salivary glands cortisol is converted to inactive cortisone by 11β hydroxy steroid dehydrogenase type 2. Brain MR is in particular involved in the regulation of basal HPA-axis pulsatility and in the onset of the stress response.

Several studies using animal models have demonstrated MR-mediated effects on behaviour. MR blockade clearly influenced coping with stressful challenges (Oitzl and de Kloet, 1992), observed as a change in the initial search strategy for an escape route in the Morris water maze. Other studies demonstrated that the MR blockade inhibits the onset of aggressive behaviour in male rats (Haller et al., 1998) and rainbow trout (Schjolden et al., 2009). Furthermore, transgenic mice with increased levels of MR in the forebrain showed decreased anxiety-like behaviour and enhanced memory (Rozeboom et al., 2007; Lai et al., 2007). In humans, MR-blockade impaired e.g. selective attention and mental flexibility, behavioural responses that represent the inital behavioural response to a stressor (Otte et al., 2007).

Involvement of genetic variation in the human MR in HPA-axis regulation is reported for two single nucleotide polymorphisms. One of these SNPs, designated MRI180V or rs5522 is located in exon 2. The SNP results in an isoleucine to valine amino acid change in the N-terminal domain of the protein and influences the transactivation capacity of the MR *in vitro* (DeRijk et al., 2006; Arai et al., 2003). MR180V is associated with enhanced cortisol, and autonomic responses to an acute psychosocial stressor measured with the TSST in young male twins (DeRijk et al., 2006). However, the enhanced cortisol response to the TSST was not found in a smaller study; instead an association between MR180V and higher ACTH responses and anxiety was observed during the

second TSST performed in this group (Ising et al., 2008). In addition, more feelings of depression have been observed in a cohort consisting of very old predominantly female individuals (Kuningas et al., 2007).

A second SNP tested in the MR is MR-2G/C or rs2070951, located two nucleotides before the first translational startsite of the MR. The C allele of the SNP creates a stronger translation site resulting in more MR protein expression compared to the G allele (this thesis chapter 3). An *in vitro* study demonstrated a change in transactivational capacity (van Leeuwen et al., 2010; Arai et al., 2003). Furthermore, the C variant of MR-2G/C is associated with lower basal cortisol levels in an elderly cohort (Kuningas et al., 2007).

In this study we aim to show involvement of the MR gene variants in neuroticism and mood changes in both controls and patients. Therefore we tested SNPs and haplotypes in the MR for associations with personality, mood and anxiety disorders in a cohort containing healthy controls and patients.

Materials and Methods

Subjects

Hundred patients (mean age 33.1 SD 11.2 years, 65 females) with a depressive and/or anxiety disorder were recruited from the outpatient department of the mental health center Rivierduinen in Leiden, the Netherlands. Fifty healthy controls (mean age 35.7 SD 14.0, 47 females) were recruited by advertisement. History of neurological or endocrine diseases, serious medical conditions, substance or alcohol abuse, pregnancy ovariectomy, psychotropic medication except a low dose of a benzodiazepine (equivalent to 30 mg oxazepam daily) and corticosteroid, estrogen, thyroid hormone, or herbal medication use were exclusion criteria. All subjects were subjected to a routine physical examination. Written informed consent was obtained from all participants. The study was approved by the ethics committee of the Leiden University Medical Center.

Psychopathological and psychological measures

All patients were diagnosed with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Diagnoses of the patients and absence op psychiatric illnesses in healthy controls was confirmed by trained interviewers using the Dutch version of the Mini International Neuropsychiatric Interview Plus 5.0.0-R (van Vliet and de Beurs, 2007; Sheehan et al., 1998).

All subjects completed the Dutch translation of the Brief Symptom Inventory (BSI) and the mood and axiety symptom questionnaire (MASQ). The BSI is a shortened version of the Symptom Checklist (SCL-90), that is used to measure psychological complaints or symptoms (de Beurs et al., 2007). The total BSI-score generates an overall measure of psychopathological symptom severity. Internal consistency of the BSI is very good (Cronbach's $\alpha = 0.96$), and validity is sufficient (de Beurs et al., 2007). The MASQ measures three scales of General Distress: depressive symptoms (12 items), anxious symptoms (11 items) and mixed symptoms (15 items), it has a anxiety-specific scale (Anxious Arousal, 17 items) and depression-specific scale (Anhedonic Depression, 22 items). The reported internal consistency for each scale is excellent with coefficient alphas ranging from

0.78 to 0.92 (Buckby et al., 2007). For both BSI and MASQ (sub)scales, higher scores reflect more symptoms.
Neuroticism was assessed with the neuroticism subscale of the NEO Five Factor Inventory (NEO-FFI)(Costa and McCrae, 1992).

Genotyping
DNA was extracted from EDTA blood samples using a QIAgen DNA blood maxi kit according to the manufacturer's protocol (QIAgen, Venlo, the Netherlands). All subjects were genotyped for rs2070951, rs5522, rs5525, rs5534, rs6535578 and rs6535579 with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAYtm methodology (Sequenom Inc., San Diego, CA, USA). Amplification reactions and parameters were based on the manufacturer's instructions and 10% of the samples were measured in duplicate. With this method it was not possible to measure rs2871 therefore SNPs rs65365578 and rs6535579 were measured instead since there is high LD between these SNPs and rs2871 accoring to the HAPMAP database. In addition, SNPs MR-2G/C and MRI180V were also genotyped using the TaqMan pre-designed SNP genotyping assays, assay ID C12007869_20 and C1594392_10 respectively, in combination with TaqMan universal PCR master mix (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands).

Statistical analysis
Haploview (Barrett et al., 2005b) was used to calculate Hardy Weinberg equilibrium (HWE) and linkage disequilibrium between the SNPs (estimated with D' and r^2). Haplotypes were estimated and assigned to each individual using SNPHAP.

All other statistical analysis was performed with SPSS 16.0 (Chicago, IL, USA). To test if the MR genotype and haplotype distribution is equal between the patient and control group X^2 tests were computed. One-way ANOVA was used to compare BSI and neuroticism scores between patients and controls and regression analysis was carried out to analyze the effects of MR haplotypes on BSI or neuroticism scores.

Results

MR genotypes and haplotypes
All samples were genotyped for the three SNPs in the coding region, rs2070951, rs5522 and rs5525 and three intronic SNPs rs2070950, rs6535578 and rs6535579 using the Sequenom MassARRAY methodology (Table 1). The exonic SNP rs2871 could not be measured therefore the two intronic SNPs rs6535578 and rs6535579 were measured instead. The two previously reported SNPs MR-2G/C and MRI180V were additionally genotyped with a Taqman genotyping assay yielding identical results. All genotypes were in Hardy Weinberg equilibrium (data not shown). LD and haplotype analyses revealed two haplotype blocks. One, containing the SNPs in the first intron and exon 2. The other, containing SNPs in exon 9 and the preceding intron (Fig. 1a and b).

Table 1. Genotype frequencies (in %) in patients and controls and the statistical comparison of genotypes between patients (n=100) and controls (n=50)

SNP	variation	homozygotes		heterozygotes		homozygotes		χ-square
		patients	controls	patients	controls	patients	controls	p-value
rs2070950	C/G	29.4	26.0	42.2	54.2	28.4	20.0	0.352
rs2070951	G/C	29.4	26.0	42.2	54.2	28.4	20.0	0.352
rs5522	G/A	77.5	78.0	18.6	22.0	3.9	0.0	0.340
rs5525	T/C	77.5	78.0	18.6	22.0	3.9	0.0	0.340
rs5534	G/A	31.4	28.0	51.9	54.0	15.7	18.0	0.886
rs6535578	A/C	46.1	38.0	42.2	46.0	12.7	14.0	0.429
rs6535579	A/G	10.8	12.0	40.2	46.0	48.0	40.0	0.506

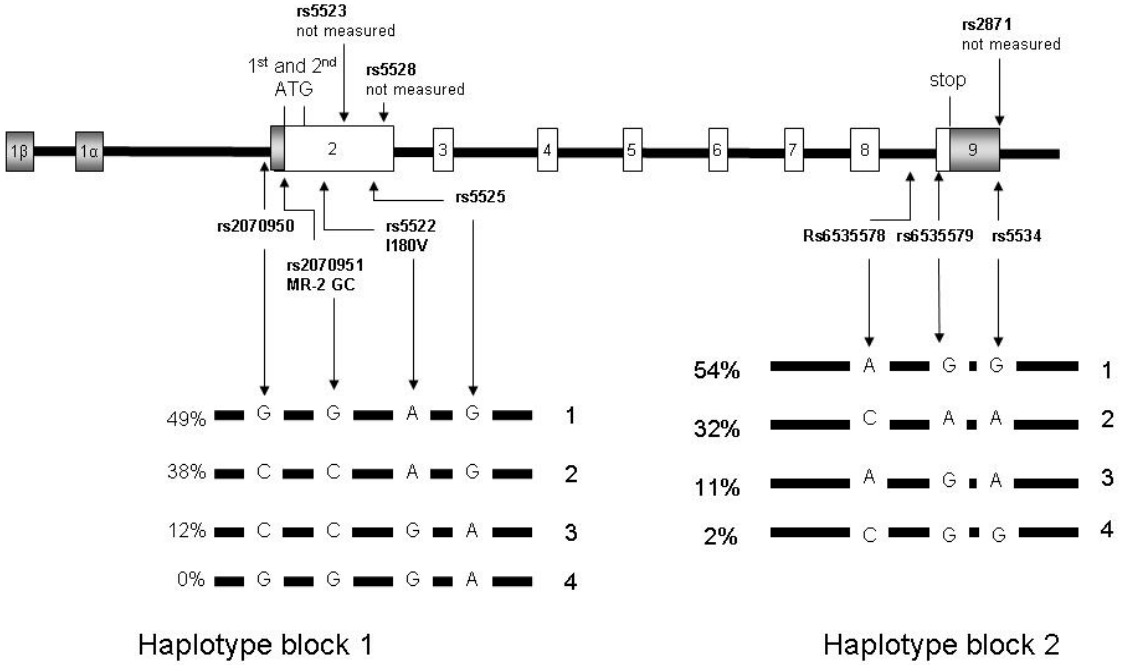
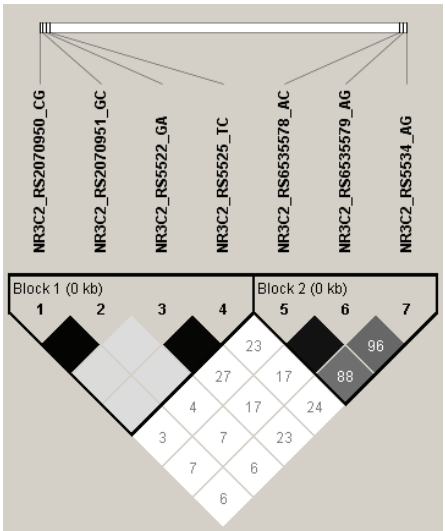


Fig. 1a. haplotypes in the MR

Fig. 1b. LD between MR SNPs measured in r^2 Black squares indicate r^2= 1 and shades of grey indicate r^2<1



Psychopathology

The genotype distribution of the seven different MR SNPs was not significantly different between the patient group and the control group (Table 2). Also the haplotype frequencies were not significantly different between the patient two groups (χ^2= 5.0 p=0.167; Table 3).

Table 3. Haplotype frequencies in patients (n=100) and controls (n=50)

	Haplotype Block 1			Haplotype Bock 2			
	1	2	3	1	2	3	4
Patients	45.9	42.9	11.2	54.8	30.1	12.0	2.0
Controls	50.0	36.7	13.4	53.1	36.7	9.2	1.0

The total score on the Brief Symptom Inventory (BSI) was significantly higher in the patients compared to the controls (patients 1.31 ±0.69 versus controls 0.18 ±0.18) and also on the eight different subscales the patients scored significantly higher (data not shown). There were no significant associations between the haplotypes and BSI total scores or subscale scores (all p>0.05), although there was a trend towards significance ($F_{1,98}$=3.34 p=0.071) that patients carrying the haplotype 2 of block 1 had a higher score on the anxiety subscale of the BSI (carriers 2.17 ±0.54 versus non-carriers 1.29 ±0.096). Also general distress, anhedonic depression, anxious arousal measured with the the mood and anxiety symptom questionnaire (MASQ) were not associated with MR haplotypes or single SNPs (all p>0.05)

Neuroticism

Neuroticism scores were significantly higher in the patient group compared to the control group (patients 44.1±7.9 versus controls 27.4±7.0) therefore both groups were analyzed separately. In the patients there was a significant association between both MR haplotype blocks and neuroticism; patients carrying one copy of allele 3 of haplotype block 1 (MR 180V carriers) had lower scores than non carriers and the scores were even lower in the patients carrying two copies of allele 3 (p= 0.04; Table. 4.). In block 2 homozygote carriers of allele 2 had lower scores of neuroticism compared to patients without allele 2 or only one copy of allele 2 (p= 0.02; Table 4.). There was no effect of the MR haplotypes on neuroticism scores in healthy individuals (p>0.05).

Table 4. Neuroticism scores in patients carrying 0, 1 or 2 copies of a haplotype

Haplotype	**Number of copies**			
Block 1	0	1	3	p
1	44.1 (±7.9)	43.6 (±8.4)	45.0 (±7.2)	n.s.
2	42. 7 (±8.0)	44.7 (±7.8)	46.3 (±7.3)	n.s.
3	45.3 (±7.4)	41.1 (±8.5)	37.3 (±7.1)	0.02
Block 2				
1	38.6 (±8.9)	45.5 (±7.6)	44.8 (±6.5)	n.s.
2	44.5 (±7.0)	45.4 (±7.3)	35.1 (±10.1)	0.02
3	44.1 (±8.1)	44.0 (±7.2)		n.s

Discussion

We described two haplotype blocks in the human MR-gene, which were associated with neuroticism but only in depressed and/or anxiety patients. However, the SNPs and the haplotype blocks in the MR gene were not associated with mood and/or anxiety disorders.

Neuroticism

Neuroticism was associated with both haplotype block 1 and 2 in the patient group; this is the first study showing that genetic variation in the MR is associated with neuroticism. Previous studies demonstrated heritability of neuroticism, associations between cortisol and neuroticism, involvement of the MR in behavior and a role for genetic variation in stress responsiveness reviewed by (Flint, 2004). The effect of the MR was only observed in the patient group, which as a total had significantly higher neuroticism scores. A depressive state amplifies the personality profile and the scores can be interpreted as an accurate reflection of the current condition of the person (Costa, Jr. et al., 2005). Therefore it is likely that the observed effect is only visible in this group with amplified scores. Another explanation why the effect is not observed in the control group might be the relative small group size (n=50) as compared to the patient group (n=100). However, also in another cohort consisting of healthy individuals from the German population we also did not observe an association between the current described MR haplotypes and neuroticism (data not shown).

Mood and anxiety

In this study we did not find an association between MR SNPs and haplotypes with mood and/or anxiety disorders. A previous study did report an association between the MR180V SNP and feelings of depression in a cohort consisting of healthy elderly (Kuningas et al., 2007). The large difference in age between the two groups might explain the discrepancy between the two findings since it is known from animal studies that MR expression decreases with aging and we demonstrated that MR haplotypes influence MR functionality and expression *in vitro* (van Leeuwen et al., 2010). It is possible that the effect of the MR haplotypes is only observed in elderly were the MR expression is probably already low. The relatively small group sizes, the patient group consisted of hundred individuals and the control group consisted of fifty individuals, might also be a reason why we did not find an association.

Mechanism

Although the mechanism of the SNPs in block 1 is partly known from previous *in vitro* experiments, it is difficult to explain the *in vivo* consequences based on these *in vitro* changes. In block 1 haplotype 3 is the allele associated with neuroticism, this is the only allele in our cohort containing the minor allele of MRI180V which leads to a lower transcriptional efficiency, without influencing the MR expression. The C allele of MR-2G/C is present in both haplotype 2 and 3 and is increasing the transactivational capacity by increasing the MR protein expression compared to the G allele of MR-2G/C present in haplotype 1(van Leeuwen et al., 2010). It seems that in block 1 MRI180V or the *in vitro* not tested SNP rs5525 causes the effect on neurotisicm. How this effect is mediated needs further investigation since both haplotypes 1 and 3 affect transactivation. In haplotype 1 this is mediated by the C allele of MR-2G/C, in haplotype 3 it is mediated by the G

allele of MRI180V while only haplotype 3 is associated with neurotiscism. Interestingly haplotype 4 which should contain both alleles that result in the lower transactivation, the G allele MR-2G/C and the G allele of MRI180V, is not observed in our population. This might occur by chance but one can speculate that this combination has negative consequences for a person's health and is therefore not passed on from generation to generation in the population.

In block 2, allele 2 is the allele associated with neuroticism. The SNPs in this block are not yet tested for functionality but computer screenings revealed that one of the SNPs rs2781 promotes loop formation of the mRNA and this will probably alter the mRNA stability. By influencing mRNA stability SNPs can increase or decrease expression of MR protein, if the mRNA is present for a longer period more protein can be formed and subsequently, more protein will lead to a higher transcriptional activity.

Furthermore, although there are no SNPs in the coding regions in the two described haplotypes blocks there are many SNPs in the intronic regions between the two blocks (described in more detail in chapter 7, the general discussion of this thesis). The intronic SNPs might affect the MR by influencing RNA splicing or transcription of the MR by changing binding of cofactors. We can not rule out that a SNP in such region is causing the effect on neuroticism.
In conclusion, there are two main haplotype blocks in the MR, these blocks were not associated with mood and/or anxiety disorders but there was an association with neuroticism in the patient group indicating involvement of the MR in human behavior.