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

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Summary

A depressive disorder is a common mood disorder characterized by loss of pleasure and a negative mood. Many people occasionally feel “depressed” but only if these feelings persist for a longer time it is possible that a person is suffering from depression. A major depression is a serious and common disease. It interferes with a person’s ability to work, study, sleep, eat and enjoy pleasurable activities. Over 15% of the Dutch population experiences depression during their life. Although many studies were performed to elucidate the etiology of the disease, the mechanism has still not been completely clarified. Stress plays an important role in the etiology of depression and some families are more vulnerable, indicating a genetic component. Twin studies in which 100% identical monozygotic twins were compared with dizygotic twins, who are on average 50% identical, confirm that there is a genetic component involved in the development of a depression.

Depression is often associated with disturbances in the hypothalamus-pituitary-adrenal (HPA) axis. The increased secretion of cortisol from the HPA axis during a stressful situation is essential for adaptation to the stressful situation. In addition, cortisol mediates inactivation of the HPA axis by negative feedback. Cortisol exerts its effect via binding to a receptor. There are two receptor types for cortisol; the mineralocorticoid receptor (MR), having a high affinity and the glucocorticoid receptor (GR), having a low affinity for the naturally occurring glucocorticoid. In the brain the MR is continuously occupied with cortisol while the GR only becomes activated at high concentrations of cortisol. Both the MR and GR play an important role in the regulation of the HPA axis and are important for behavioral adaptation.

The ultimate goal of the research described in this thesis was to investigate whether genetic variation in the mineralocorticoid receptor is involved in the development of depression.

First, the coding region of the MR gene was screened for genetic variation and using computer programs linkage between the genetic variation and the possible functionality of the genetic variation was tested; this is described in **chapter 2**. There were seven Single Nucleotide Polymorphisms (SNPs) located in the coding region of the MR-gene. A SNP is a variation in one nucleotide with a frequency of more than 1% in the population. The SNPs in the MR gene are mostly linked and several SNPs are putatively functional. The putative functional SNPs are MR-2G/C (rs20170951), MRI180V (rs5522) and rs2871. MR-2G/C is located just outside the coding region of the gene in the Kozak region and the SNP might therefore influence the translation of the mRNA to protein. MRI180V is located in the coding region of the gene in exon 2 and changes the amino acid on position 180 from an isoleucine to a valine. Rs2871, one of the SNPs in the non-coding domain of exon 9, is located in a part of the mRNA that is prone to loop formation and this might have consequences for the stability of the mRNA.

Based on the location and putative function, the SNPs MR-2G/C and MRI180V were selected for further research. Functionality of these SNPs was tested with *in vitro* cell systems. The results of the *in vitro* tests are described in different chapters of this thesis. The C allele of MR-2G/C results in

more MR protein compared to the G allele, while the amount of MR mRNA was not influenced, this was described in **chapter 4**. The increased MR expression with the C allele of MR-2G/C lead to higher transactivation as descibed in **chapter 3 and 4** and increased ligand binding, described in **chapter 5**. MRI180V had previously been tested in transactivation assays, the V allele decreased transactivation without changing the expression or ligand binding (**chapter 5**).

MR-2G/C and MRI180V were, to a large extent, linked to each other, as described in **chapter 2, 3, 5 and 6**. The combination of two (partly) linked SNPs is called a haplotype. Without linkage 4 different combinations (haplotypes) are possible. In our studies, however, only 3 combinations (haplotypes) were observed. The combination MR-2G and MR180V (=G nucleotide) did not occur. To exclude the possibility that the two SNPs influence each other's functionality, the haplotypes were also tested *in vitro*, this was described in **chapter 5**. The two SNPs did not influence each other *in vitro*.

In each chapter an association study was performed between genetic variation and one of the possible consequences of the variation.

In **chapter 3** involvement of the two *in vitro* functional SNPs MR-2G/C and MRI180V on the regulation of the HPA axis in healthy individuals was tested by using the dexamethasone suppression test (DST). Dexamethasone is a synthetic glucocorticoid and, like high concentrations of cortisol, it is able to suppress the HPA axis. In our study a low dose dexamethasone (0.25mg) was used and this resulted in decreased but not completely suppressed cortisol levels the day after intake. The peak in cortisol lasting one hour just after awakening was not influenced by the SNPs without dexamethasone. However, after the intake of dexamethasone the previous evening there was a clear effect of the SNPs on cortisol levels and this effect was gender specific. The decrease was highest in women with the MR-2G/C GG genotype, while men with this genotype showed an increase compared to the men with other genotypes. Men with the MRI180V AA genotype had higher cortisol concentrations than men with the AG genotype, while this SNP had no effect in women. Haplotype analysis did not reveal additional information; the effect was mainly mediated by MR-2G/C.

In **chapter 4** the influence of MR-2G/C on blood pressure and salt regulation was tested. In addition to the important role of the MR in the HPA axis, the MR is also important in de regulation of salt and blood pressure via the renine angiotensine system (RAS). The hormone aldosterone binds the MR receptor in the kidney and this results in water and salt retention The G allele of MR-2G/C was associated with increased activation of the RAS and higher blood pressure.

In **chapter 5** the influence of the MR haplotypes containing MR-2G/C and MRI180V on the response to an acute psychosocial stressor and on chronic stress was tested. Individuals homozygous for the haplotype containg the MR-C and MRI180 alleles had the highest saliva cortisol, plasma ACTH and heart rate during the psychosocial stressor. This haplotype resulted in vitro in the highest MR expression and transactivation. Chronic stress was measured with questionaires and the subscales "social isolation" and "work overload" were associated with the MR haplotypes, however this was mediated by the haplotype with the MR-C and MR180V alleles.

Individuals carrying this hapotype reported more chronic stress. The set-up of this study was hypothesis generating; the results suggest that more MR protein leads to higher responses on acute psychosocial stress and that MR haplotypes influence the perception of chronic stress.

In **chapter 6** two MR haplotypes were tested and described in a cohort consisting of one hundred patients with mood and/or anxiety disorders and fifty healthy individuals. Due to the relatively low number of patients and controls this study was also explorative in nature and hypothesis generating. Association studies were performed with haplotypes in the beginning of the gene (5', -2 G/C en 180) and with haplotypes at the end of the gene (3', in exon 9). The two haplotypes were not associated with mood and/or anxiety disorders but in the patient group there was an association with the personality trait neuroticism.

Chapter 7 is the general discussion of the thesis.
First, the location of the SNPs was analyzed. MR SNPs were not located throughout the gene, they were mainly located in the promoter region, exon 2 and at the end of exon 9. Genetic changes on other locations are probably too severe to be spread through the population.
Secondly, the predictive value of the *in vitro* studies was discussed. The *in vitro* functional SNPs were associated with different measures *in vivo,* therefore it seems important to perform in vitro studies. However, generating a detailed hypothesis for *in vivo* associations based on the *in vitro* results appeared to be complicated. This is illustrated by the gender differences *in vivo*. Many associations were gender specific, the effects being different in men and women. Some associations were in the completely opposite direction between men and women. In the current *in vitro* assays there are no sex hormones present. Adding sex hormones in the *in vitro* studies or testing different age groups *in vivo* might clarify the associations. These and other recommendations for future studies are described in **chapter 7**.

In this thesis the genetic variation in the MR was described. There is genetic variation in the MR and this variation is functional in *in vitro* studies. The genetic variation was associated with cortisol levels after dexamethasone, the reaction on psychosocial stress, chronic stress and neuroticism. Due to the relatively low number of individuals in each study, these studies were explorative and the associations found need follow up testing. We postulate that genetic variation in the MR modulates the stress response and affects in part a person's personality. Furthermore, the genetic variant MR-2G/C was associated with blood pressure and salt regulation and we were able to confirm this in different cohorts.