Mineralocorticoid receptor in human brain : a key player in resilience

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Chapter 7

A common and functional mineralocorticoid receptor gene haplotype associates with a lower risk of major depressive disorder in females

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Abstract

Background: Through the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) in the brain the steroid hormone cortisol promotes adaptation to stress and health. Impaired adaptation to stress enhances the vulnerability to major depressive disorder (MDD) and is characterized by disturbances in the regulation of circulating cortisol. Therefore, an imbalance in MR versus GR activity is thought to be implicated in the pathophysiology of MDD. We have repeatedly been able to show a link between *MR* gene variants and variability in circulating cortisol, psychological traits and symptoms of depression. The purpose of the present study was to extend and validate those results by performing an association study between the functional *MR* gene variants and MDD diagnosis among a large cohort of MDD patients and healthy controls.
Methods: The common and functional 5' *MR* haplotypes 1 (-2G/180I; freq. .50), 2 (-2C/180I; freq .38) and 3 (-2C/180V; freq .12) were tested for association with MDD using data from a large Dutch genome-wide association (GWA) study (GAIN-MDD). MDD cases (n= 1730) were mainly from the Netherlands Study of Depression and Anxiety (NESDA) and control subjects (n= 1793) were mainly from the Netherlands Twin Registry (NTR).
Results: In line with previous results, *MR* haplotype 2 associated with a lower risk of depression, specifically in women. Interestingly, the association was particularly found in women aged 41 years or younger.
Conclusion: To conclude, the *MR* gene is a significant modulator of psychological health, making it an important target for MDD prevention and treatment among women.

Keywords: mineralocorticoid receptor, single nucleotide polymorphism, haplotype, depression, sex difference

Introduction

Major depressive disorder (MDD) is a common disorder with a lifetime prevalence of around 15% in the Dutch population, striking women twice as often as men (Bijl et al., 1998). Clearly, environmental factors as well as genetic factors are involved in the pathophysiology of depression. Numerous studies suggest a causal relationship between stressful (environmental) experiences like the death of a spouse and the development of depression (Kendler et al., 1999; de Graaf et al., 2002; Tennant, 2002; Spinhoven et al., 2010). However, while many individuals are able to cope with adverse experiences, others may succumb. Whether a person is susceptible to stress and psychopathology depends to a large extent on a subject's genetic makeup. The chance to get depressed is higher when psychiatric disturbances are prevalent in the family. Twin studies indicate that the heritability of MDD is around 30-40% (Sullivan et al., 2000). Unfortunately, researchers experience difficulties in consistently identifying genetic risk factors. It is now recognized that environmental and genetic factors may interact in tipping the balance from resilience to vulnerability (Caspi et al., 2010).

Ample lines of evidence suggest the implication of the central mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in the development of depression (de Kloet et al., 1998; Holsboer, 2000). Both the MR and GR are receptors for cortisol and modulate activity of the hypothalamic-pituitary-adrenal (HPA) axis (Reul and de Kloet, 1985), which is often disturbed in depression (Holsboer, 2000). Cortisol affects many functions in the periphery and in the brain, among others learning and memory processes and emotional arousal (de Kloet et al., 2005). Therefore, an imbalance in central MR vs. GR activity may underlie HPA axis disturbances and problems with emotions and cognition. Manipulation studies in rodents and humans have identified particularly the MR to be important for stress appraisal, behavioral response selection and emotional arousal (Oitzl et al., 1994; de Kloet et al., 2005; Berger et al., 2006; Brinks et al., 2007b; Otte et al., 2007).

In order to extend those results related to the MR to the human situation, we have performed multiple genetic association studies among various groups of healthy subjects and patients. We have been able to show that common single nucleotide polymorphisms (SNPs) at the 5' end of the *MR* gene (including the -2G/C and I180V SNPs and several SNPs in the gene's promoter region, see **Chapter 2**) affect *MR* transcription, translation and its capacity to transactivate target genes (as after binding its ligand the MR acts as a transcription factor) (van Leeuwen et al., 2010a, 2010b, 2011) (**Chapter 3**). These functional SNPs modulate HPA activity under basal (non-stress) conditions and in response to stress (DeRijk et al., 2006; van Leeuwen et al., 2010a; van Leeuwen et al., 2011) (**Chapter 6**). Moreover, the functional SNPs associate with variability in personality traits (**Chapter 4** and **5**), experience of stress at work (van Leeuwen et al., 2011) and reward learning under stress (Bogdan et al., 2010). Finally, some first indications exist for a relation between the *MR* genotype and symptoms of depression (Kuningas et al., 2007) (**Chapter 5**). Together the data suggest that a 5' *MR* haplotype (consisting of the -2 C-allele and the 180 I-allele), with a frequency of ~36% in the population, that results in the highest MR activity (*in vitro*) predicts a lower risk of depression, particularly in females.

In order to validate and strengthen the results thus far, we performed an association study between the common 5' *MR* haplotypes and MDD diagnosis using data of a large case-control study. The current study supports our previous evidence that *MR* haplotype 2 is associated with a lower risk of depression, specifically among women.

Methods

Study population

To test association of the *MR* haplotypes with MDD, data were used from a large GWA study, the GAIN-MDD study (Sullivan et al., 2009). MDD cases (n= 1730) were mainly from the Netherlands Study of Depression and Anxiety (NESDA; http:// www.nesda.nl) (Penninx et al., 2008). The NESDA study is an eight-year longitudinal cohort study on the causes and course of depressive and anxiety disorders in people aged 18-65 years. The patients included here had a lifetime diagnosis of MDD as diagnosed with the DSM- IV Composite International Diagnostic Interview (CIDI) version 2.1. The control subjects (n= 1793) were mainly from the Netherlands Twin Registry (NTR; http://www.tweelingenregister.org), which is a longitudinal study that collects data from twins and their families since 1991 (Boomsma et al., 2006). The control subjects included here had no report of MDD, as determined by specific queries about medication use or whether the subject had ever sought treatment for depression symptoms and/or through the CIDI interview. For further details on the inclusion and exclusion criteria for the GAIN-MDD study see (Sullivan et al., 2009) (see **Table 1** for sample characteristics). The NESDA and NTR studies were approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam, The Netherlands. All subjects provided written informed consent.

DNA sampling and genotyping

For the patients as well as the control subjects DNA was isolated from blood and genotyping was performed by Perlegen Sciences (Mountain View, CA, USA) using the Perlegen GWAS platform (for details on DNA isolation and genotyping see (Sullivan et al., 2009)). In the present study we used the genotypes for the functional *MR* -2G/C (rs2070951_GC) and I180V (rs5522_AG) SNPs, which tag the three most common haplotypes localized in exon 2 and extending into the promoter region (see also **Chapter 3**).

Statistical analysis

SNP allele frequencies in the control group were tested for Hardy-Weinberg equilibrium (HWE) using HaploView (version 4.1 for Mac OS X; available online at http://www.broadinstitute.org/mpg/haploview) (Barrett et al., 2005). In addition, HaploView was used to assess inter-marker LD scores (expressed as *D'* and r^2) between the *MR* SNPs and to reconstruct haplotypes. Individual haplotypes were reconstructed in SNPHAP (version 1.3; available online at http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt). Differences between men and women in age, SNP or haplotype frequencies, or the percentage of subjects included in the depressed or control group were tested using a χ^2-test or a non-parametric Mann-Whitney *U*-test, where appropriate.

Association between the single SNPs and MDD diagnosis was tested with logistic regression. For association analysis with the haplotypes, three dummy variables were created indicating whether a person carried zero, one, or two alleles of haplotype 1, 2, or 3. The mean effect of a haplotype 2- or 3-allele on the risk of depression was determined relative to the reference group (haplotype 1 carriers). Based on the previous data showing clear sex differences, regression analyses were repeated in sex strata. Finally, because sex-dependent associations are potentially due to differences in circulating sex steroids, the women were additionally split for the mean age for menopause (~51 yrs). A two-sided *p*-value < .05 was considered statistically significant. A Bonferroni correction was applied where appropriate. Statistical analysis was performed using SPSS, version 16.0 for Mac OSX (SPSS Inc., Chicago, IL, USA).

Results

Sample characteristics, genotype and haplotype frequencies

The percentage of women included in this study was significantly higher ($p < .001$, **Table 1**), while the women were significantly younger compared to the men ($p < .001$). In addition, the group of women included more subjects carrying a I180V 'AA' genotype and fewer subjects carrying a 'AG' genotype compared to the men ($p = .008$). Allele frequencies of the *MR* SNPs in the control group were in HWE ($p > 0.20$). Genotype and haplotype frequencies and inter-marker correlations between the *MR* -2G/C and I180V SNPs ($D' = 1.0$; $r^2 = 0.14$) were similar as previously found.

Table 1 Subject characteristics and *MR* SNP and haplotype frequencies according to sex in the patients with major depressive disorder (n= 1730) and healthy controls (n= 1793)

Variable	Total	Women	Men	*p*-value
	n= 3523	n= 2312 (65.6%)	n= 1211 (34.4%)	
Age, mean yrs (SD) #	43.9 (13.4)	42.7 (13.2)	46.1 (13.4)	**< .001**
Major depressive disorder, %	49.1	52	43.6	**< .001**
MR variants				
-2G/C, GG/CG/CC, freq.	.23 / .53 / .24	.24 / .53 / .23	.22 / .52 / .26	.23
I180V, AA/GA/GG, freq.	.77 / .21/ .02	.78 / .20 / .02	.74 / .24 / .02	**.008**
MR hap 1 -2G/180A, freq.	.50	.50	.48	.09
MR hap 2 -2C/180A, freq.	.38	.38	.38	
MR hap 3 -2C/180G, freq.	.12	.12	.14	

Notes: Significant *p*-values are indicated in bold. # Mann-Whitney *U*-test.

Associations between the individual *MR* SNPs and diagnosis of depression

No association was found between the single -2G/C or I180V SNP and diagnosis of depression, not in the total group, nor in the women or men separately (**Table 2**). However, when the women were split for the mean age for menopause (~51 years) a trend ($p = .08$) was found for an association between the -2G/C SNP and diagnosis of depression

according to a dominant model (similar results were found with a linear model but showed smaller effect sizes). Explorative analysis revealed that the association was present particularly in women with an age ≤ 41 years (0.75; 95% confidence interval= 0.57-0.99; *p*= .04).

Table 2 Results of logistic regression analysis associating the function *MR* -2G/C and I180V SNPs with MDD diagnosis, regression coefficients (*B*), standard errors (*SE*), *p*-values, odds ratios and 95% confidence intervals

	-2G/C (rs2070951) GC + CC vs. GG				I180V (rs5522) AG + GG vs. AA			
	B	*SE*	*p*	*odds ratio (95% CI)*	*B*	*SE*	*p*	*odds ratio (95% CI)*
Total	-0.06	0.08	.46	0.94 (0.81-1.10)	-0.06	0.08	.41	0.94 (0.80-1.10)
Women	-0.08	0.10	.43	0.93 (0.76-1.12)	0.01	0.10	.88	1.01 (0.83-1.24)
Men	0.00	0.14	1.0	1.00 (0.76-1.31)	-0.16	0.13	.22	0.85 (0.65-1.10)
Women <= 51 yrs	-0.21	0.12	.08	0.81 (0.64-1.02)	0.09	0.12	.48	1.09 (0.86-1.38)
Women > 51 yrs	0.20	0.18	.25	1.22 (0.87-1.72)	-0.25	0.19	.19	0.78 (0.54-1.14)
Women <= 41 yrs	-0.29	0.14	**.04**	0.75 (0.57-0.99)	0.16	0.14	.27	1.17 (0.89-1.54)
Women > 41 yrs	0.12	0.12	.36	1.13 (0.87-1.48)	-0.13	0.14	.38	0.88 (0.66-1.17)

Notes: Effects for 1 or 2 minor alleles were calculated as compared to the reference group (major allele carriers). Data are presented for the total group, for women and men separately and for women of different age groups. The -2 C-allele associated with MDD among women according to a dominant model, particularly in women aged ≤ 41 yrs. Significant *p*-values are indicated in bold.

MR haplotype 2 associates with a lower risk of major depressive disorder, specifically in women

While adjusting for age, a trend was found for an association between *MR* haplotype 2 and a lower odds ratio for MDD in women (0.85; 95% confidence interval= 0.72-1.02; *p*= 0.08) and not in men (*p*= 0.72), again according to a dominant model (**Figure 1A** and **Table 3**; similar results were found with a linear model but showed smaller effect sizes). However, a strong association was found between *MR* haplotype 2 and a lower odds ratio for MDD in the women with an age ≤ 51 yrs (0.75; 95% confidence interval= 0.60-0.93; *p*= 0.009; after a Bonferroni correction for in total four tests for association analysis within both sexes and within the two age groups, with a significance threshold of *p*< 0.0125, this is still significant; **Figure 1B**). Explorative analysis revealed that the association particularly existed in the women with an age ≤ 41 yrs (0.66; 95% confidence interval= 0.52-0.86; *p*= 0.002; **Figure 1C**). **Figure 2** shows the number of subjects diagnosed with depression according to the *MR* diplotypes, which makes clear that the *MR* haplotype 2 has a dose, but dominant, effect.

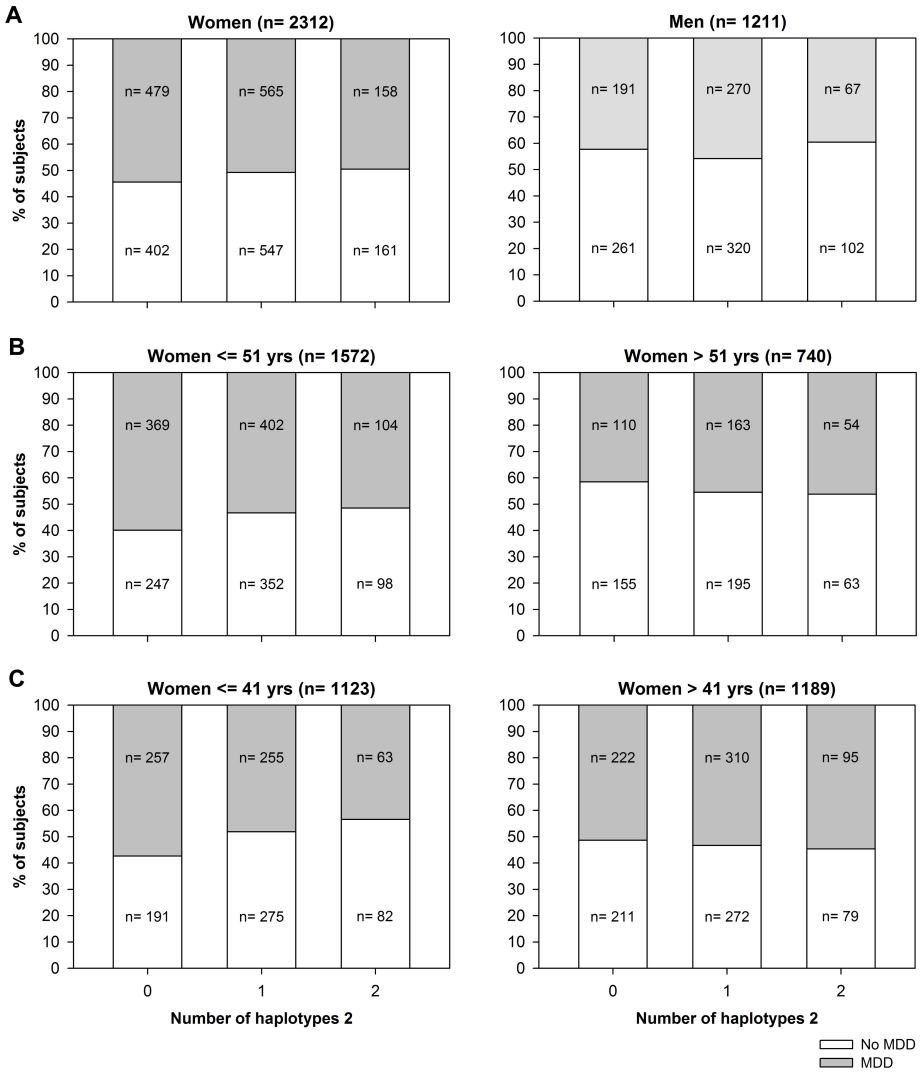

Figure 1 Percentage of subjects diagnosed with MDD according to the number of haplotypes 2. Results are presented separately for women and men **(A)**, for the women aged ≤ 51 yrs versus > 51 yrs **(B)** or for the women aged ≤ 41 yrs versus > 41 yrs **(C)**.

Table 3 Results of logistic regression analysis associating three 5' *MR* haplotypes with MDD diagnosis, regression coefficients (*B*), standard errors (*SE*), *p*-values, odds ratios and 95% confidence intervals

		MR hap 2 1 or 2 hap 2 alleles vs. 0				*MR* hap 3 1 or 2 hap 3 alleles vs. 0			
		B	*SE*	*p*	*odds ratio (95% CI)*	*B*	*SE*	*p*	*odds ratio (95% CI)*
Total	Unadjusted	-0.10	0.07	.17	0.91 (0.79-1.04)	-0.10	0.08	.24	0.91 (0.77-1.07)
n= 3523	Adjusted	-0.09	0.07	.22	0.92 (0.79-1.06)	-0.09	0.08	.31	0.92 (0.78-1.08)
Women	Unadjusted	-0.17	0.09	.07	0.85 (0.71-1.01)	-0.04	0.11	.69	0.96 (0.78-1.18)
n= 2312	Adjusted	-0.16	0.09	.08	0.85 (0.72-1.02)	-0.05	0.11	.61	0.95 (0.77-1.17)
Men	Unadjusted	0.05	0.13	.68	1.05 (0.82-1.34)	-0.15	0.14	.29	0.86 (0.66-1.13)
n= 1211	Adjusted	0.05	0.13	.72	1.05 (0.82-1.34)	-0.14	0.14	.33	0.87 (0.66-1.15)
Women <= 51 yrs (n= 1572)		-0.29	0.11	**.009**	0.75 (0.60-0.93)	-0.02	0.13	.89	0.98 (0.77-1.26)
Women > 51 yrs (n= 740)		0.13	0.16	.44	1.13 (0.83-1.53)	-0.21	0.20	.30	0.81 (0.55-1.20)
Women <= 41 yrs (n= 1123)		-0.41	0.13	**.002**	0.66 (0.52-0.86)	0.01	0.15	.96	1.01 (0.75-1.35)
Women > 41 yrs (n= 1189)		0.07	0.13	.59	1.07 (0.84-1.37)	-0.11	0.15	.48	0.90 (0.67-1.21)

Notes: Effects for 1 or 2 alleles of haplotype 2 or 3 were calculated as compared to the reference group (haplotype 1 carriers/subjects carrying no haplotype 2 or 3). Data are presented for the total group, for women and men separately and for women of different age groups. Adjusted results were corrected for sex (in the total group) and age. Haplotype 2 associated with MDD among women according to a dominant model, particularly in women aged ≤ 41 yrs. Significant *p*-values are indicated in bold.

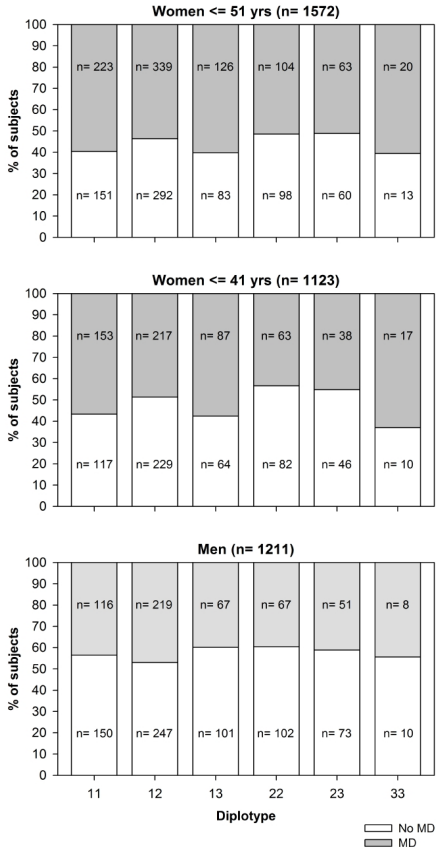

Figure 2 Percentage of subjects diagnosed with MDD according the six 5' *MR* diplotypes. Results are presented separately for **(A)** women aged ≤ 51 yrs, for **(B)** women aged ≤ 41 yrs and for **(C)** all men.

Discussion

We have previously shown that a common (frequency ~ 0.38) *MR* gene variant, *MR* haplotype 2, results in the highest MR activity *in vitro* and a better psychological condition *in vivo*. Here we show that this same *MR* gene variant associates with a lower risk of depression in a large case-control study. Results were restricted to women with a strong association found particularly in women aged 41 years or younger. Together the data indicate that the MR is an important modulator of psychological health in women.

Our data confirm the hypothesis that the MR plays an important role in depression. Although it may be difficult to model depression in rodents, various studies indicate that the MR modulates emotions like fear and anxiety (Brinks et al., 2007b; Rozeboom et al., 2007). In depressed patients HPA axis activity is often disturbed (Holsboer, 2000) and HPA normalization seems to predict clinical relief (Zobel et al., 2004), while persisting disturbances may predict relapse (Appelhof et al., 2006). This suggests that this system and its regulators may be involved in the pathophysiology of depression. A few studies indicate that MR (and GR) expression is disturbed in the brain of depressed patients (Lopez et al., 1998; Xing et al., 2004; Wang et al., 2008) (**Chapter 2**) while rodent studies show that long-term administration of antidepressants induces hippocampal MR (and GR) expression (Seckl and Fink, 1992; Bjartmar et al., 2000). Moreover, MR agonists and MR antagonists enhance or suppress the efficacy of antidepressants respectively (Holsboer, 1999; Otte et al., 2010). We have used *MR* genetic variants as a tool to demonstrate a relation between the MR and depression and indeed a specific *MR* genetic variant with a high frequency in the population was significantly associated with a lower risk of depression in females.

Again, only in women the *MR* gene was found to modulate the risk of depression, particularly in the women aged 41 years or younger. This suggests that female sex steroids interact with the *MR* gene in the modulation of resilience. The *MR* haplotypes are known to confer differences in MR activity with varying ligand availability (van Leeuwen et al., 2011), while HPA responses to stress are gender-specific (Kudielka and Kirschbaum, 2005). Moreover, estrogens and androgens modulate MR mRNA and/or protein expression while progesterone is able to bind the MR, with possible consequences for stress-reactivity (Carey et al., 1995; Castren et al., 1995; Turner, 1997; Quinkler et al., 2002). However, no significant interaction effect was found between the haplotypes and the use of oral contraceptives (data not shown). Still, this age difference may also be due to a difference in pathophysiology; the impact of genetic factors and HPA axis disturbances appears to be larger for early-onset vs. late-onset depression (Kendler et al., 2009).

The mechanism through which glucocorticoids and the MR modulate psychological risk of depression remains unclear. People at risk of depression are thought to cope less efficient with challenges at a neuroendocrine and behavioral level (Southwick et al., 2005). We propose the mechanism through which the *MR* gene modulates psychological risk of depression to implicate the reward system, influencing a subject's behavioral flexibility and vulnerability for distress. Indeed, through the MR and GR, glucocorticoids are known to act on the brain reward system (de Jong and de Kloet, 2004; Fiancette et al., 2010).

Concordantly, in a recent study *MR* 180 V-allele carriers seemed to be less able to modulate behavior as a function of reward after an acute, uncontrollable stressor (Bogdan et al., 2010).

Although the results are limited by the fact that stressful life experiences were not taken into account, the present results confirm earlier results showing this same *MR* haplotype 2 to associate with heightened dispositional optimism in one study (**Chapter 4**) and with less hopelessness, rumination and a lower risk of self-reported diagnosis of depression in another study (**Chapter 5**). Together the data suggest that the *MR* genotype is an important factor involved in inter-individual differences in psychological resilience to stress-related psychopathology. The results call for longitudinal studies examining whether the *MR* haplotype 2 provides resilience to mental disorders. This may also help to elucidate why women may be more vulnerable to the effect of stressful life events (Maciejewski et al., 2001; Vahtera et al., 2006). Future studies should establish whether the MR is a target for the prevention and treatment of psychiatric symptoms.

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