Mineralocorticoid receptor in human brain : a key player in resilience

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Chapter 5

A functional mineralocorticoid receptor gene haplotype associates with decreased cognitive reactivity to sad mood in young women not men

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Accepted for publication in Translational Psychiatry together with Chapters 3, 4 and 7.

Abstract

Background: The effects of cortisol on behavior and cognition are mediated by central functioning of the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). A balanced activity of the MR together with the GR is thought to be crucial for an individual to cope with stress and for resilience against psychopathology. Evidence from animal and human studies indicates that when confronted with a challenge, mainly the MR is important for behavioral flexibility. We hypothesize that *MR* gene variants influence psychological resilience against depression. Indeed, a common *MR* single nucleotide polymorphism (SNP; I180V) was found to be associated with feelings of depression in elderly, while a common and functional *MR* haplotype (haplotype 2, including the -2 C-allele and the 180 I-allele) was found to be associated with heightened dispositional optimism among female participants of the Arnhem Elderly Study.
Methods: In the present study we tested the association between the *MR* -2G/C and I180V single nucleotide polymorphisms (SNPs) and their respective haplotypes and cognitive reactivity to sad mood, in particular thoughts of hopelessness. Hopelessness may increase the risk of depression and is often present in depressed patients. A group of 150 students (44 M/106 F; 23.9±5 yrs) completed the Leiden Index of Depression Sensitivity-Revised (LEIDS-R) questionnaire, a measure of cognitive reactivity to sad mood. In addition, subjective levels of neuroticism were assessed as well as symptoms of depression and anxiety and self-reported diagnosis of depression.
Results: In line with the previous data, a significant association was found between *MR* haplotype 2 and less thoughts of hopelessness, only in women ($p = .04$) and not in men ($p = .78$). Moreover, haplotype 2 also related to lower scores of the women on the LEIDS-R aggression ($p < .01$), risk aversion ($p = .05$) and rumination ($p < .001$) subscales, neuroticism ($p = .03$) and to a lower frequency of self-reported depression ($p = .03$).
Conclusion: Together the data suggest that genetic variability in the *MR* gene modulates psychological vulnerability traits in women, potentially influencing the risk of depression.

Key words: mineralocorticoid receptor, single nucleotide polymorphisms, haplotypes, cognitive reactivity, vulnerability, depression

Introduction

The mineralocorticoid receptor (MR) has a crucial function in the brain. Being one of the two receptors for cortisol (besides the glucocorticoid receptor, GR) it regulates ultradian and diurnal activity of the hypothalamic-pituitary-adrenal (HPA) axis and mediates effects of cortisol on cognition and behavior (de Kloet et al., 2005). Studies with rodents have shown that the MR is important for behavioral flexibility when confronted with novel situations and it confers increased explorative behavior and less anxiety (Oitzl et al., 1994; Conrad et al., 1997; Berger et al., 2006; Brinks et al., 2007b). That the MR plays a role in human cognitive flexibility has been described as well. Blockade of the MR impairs selective attention and visuospatial memory (Otte et al., 2007). This raises the possibility that *MR* gene variants affect the role of the MR in behavior, cognitive processes and potentially psychopathology.

Two single nucleotide polymorphisms (SNPs) in the *MR* gene have been frequently addressed, the *MR* -2G/C and I180V. The combination of the -2 C-allele and the 180 I-allele (haplotype 2, freq. ~0.42) results in higher MR protein expression and capacity to activate target genes *in vitro* (van Leeuwen et al., 2011). This haplotype 2 extends into the promoter region, where multiple SNPs confer higher *MR* gene transcription (**Chapter 3**). These functional SNPs associate with inter-individual variability in cortisol levels, particularly in combination with a 'challenge' (e.g. dexamethasone treatment, exposure to a psychosocial stressor (DeRijk et al., 2006; Kuningas et al., 2007; van Leeuwen et al., 2010a; van Leeuwen et al., 2011). A first indication for a possible relationship with depression came from a study among elderly, showing that the *MR* 180 V-allele associated with more feelings of depression (Kuningas et al., 2007). Together the data suggest that *MR* gene variants may influence an individual's risk of depression through modulation of behavioral and cognitive flexibility.

In a first attempt to address this hypothesis, we were able to show that *MR* haplotype 2 associated with heightened dispositional optimism (**Chapter 4**), a trait that has been described to decrease the risk of depression (Vickers and Vogeltanz, 2000; Giltay et al., 2006a). Important to note is that the associations found with the *MR* gene variants often differ among the sexes. Only in women optimism scores depended on *MR* genotype, which is of interest considering the two times higher prevalence of depression among women compared to men (Bijl et al., 1998). Here we report on a follow-up study, focusing on cognitive reactivity to sad mood. Cognitive reactivity has been described as the ease with which small non-pathological changes in mood trigger maladaptive cognitions (Scher et al., 2005). Maladaptive cognitions, for example repetitive thoughts about negative emotions (rumination), are thought to play a significant role in the onset and maintenance of depression (Nolen-Hoeksema, 2000). After recovery from a depressive episode these dysfunctional thoughts do not just seem to disappear but become latent. Experimental studies using sad mood inductions have shown that formerly depressed patients show higher cognitive reactivity than never-depressed individuals (Segal et al., 1999), while high cognitive reactivity among recovered patients increases the risk of depressive relapse, independently from prior treatment (Segal et al., 2006). Cognitive reactivity seems to depend highly on childhood trauma, in part through interaction with the serotonin transporter

gene (*5-HTT*), providing new evidence for the involvement of gene-environment interactions in psychological health (Antypa and Van der Does, 2010b). Here we specifically concentrated on thoughts of hopelessness as opposed to dispositional optimism. Hopelessness may increase the risk of depression and is often present in depressed patients (Lakdawalla et al., 2007).

We propose that the MR modulates specific aspects of psychological functioning that are underlying psychological health. As a follow-up on the association found with dispositional optimism we tested whether the *MR* gene variants related to variability in an opposing trait, namely thoughts of hopelessness when in a low mood. Levels of hopelessness were measured among 150 students as part of the Leiden Index of Depression Sensitivity-Revised (LEIDS-R) (Van der Does, 2002). The LEIDS-R is a self-report measure of cognitive reactivity and contains six subscales that cover several dimensions of maladaptive cognitions. It captures individuals that have a history of depression and correlates with cognitive reactivity as measured with a mood induction procedure. Because of the clear sex-difference observed in the previous study on the association between the *MR* haplotypes and dispositional optimism, analysis was stratified for sex. Finally, as effects of the *MR* gene variants often become apparent in combination with a 'challenge' we verified whether the associations found were moderated by childhood abuse.

Methods

Study population

The study population consisted of 150 university students (see **Table 1** for sample characteristics). All participants gave written informed consent. This study was approved by the Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands). An enlarged study group (n= 250) was used for association analysis with a serotonin transporter polymorphism (5-HTTLPR) (Antypa and Van der Does, 2010b).

Questionnaires

Hopelessness was measured as part of the Leiden Index of Depression Sensitivity-revised (LEIDS-R), a measure of cognitive reactivity to sad mood (Van der Does, 2002). It is a self-rating questionnaire consisting of 34 statements (totLEIDS) constituting six subscales, namely *Hopelessness/Suicidality* (HOP; e.g., "When I feel down, I more often feel hopeless about everything"), *Acceptance/Coping* (ACC; e.g., "When in a sad mood, I feel more like myself"), *Aggression* (AGG; "When I feel down, I lose my temper more easily"), *Perfectionism/ Control* (PFC; e.g., "When in a sad mood, I become more bothered by perfectionism"), *Risk Aversion* (RAV; e.g., "When in a low mood, I take fewer risks") and *Rumination* (RUM; e.g., "When I feel sad, I spend more time thinking about the possible causes of my moods"). Participants have to indicate whether and how their thinking patterns change when they experience mild dysphoria, by scoring each item on a 5-point Likert-scale ranging from 0 "not at all" to 4 "very strongly". Scores for the subscales range from 0 to 20 for *Hopelessness/Suicidality* and *Acceptance/Coping* and from 0 to 24 for *Aggression*, *Perfectionism/ Control*, *Risk Aversion* and *Rumination*. Higher scores indicate higher

cognitive reactivity. In the complete sample of 250 students internal consistency (Cronbach's α) for the total LEIDS-R was 0.89 and ranged between 0.62 and 0.83 for the subscales.

The Dutch/Flemish version of the 60-item Neuroticism-Extraversion-Openness Five Factor Inventory (NEO-FFI, which is a short version of the NEO Personality Inventory Revised, NEO-PI-R) was used to assess neuroticism (Hoekstra et al., 2007). Its scores may range from 12 to 60. Internal consistency for the complete sample was α= 0.88. Current symptoms of depression and anxiety were assessed with the Dutch version of the Hospital Anxiety and Depression Scale (HADS), a 14-item self-report questionnaire (Spinhoven et al., 1997). Scores for the total scale may range from 0 to 42. In the complete sample Cronbach's α for the total scores was 0.87. The major depression questionnaire (MDQ) was used to assess presence of current or past depression (Van der Does et al., 2003). It is a self-report questionnaire consisting of 18 questions that covers all diagnostic criteria of the DSM-IV. Consistency of this questionnaire with diagnoses based on SCID interviews was previously examined (Williams et al., 2008). Trauma during early life was assessed with the 28-item version of the Childhood Trauma Questionnaire (CTQ-SF) (Bernstein and Fink, 1998). This questionnaire has the property to measure history of maltreatment in clinical as well as non-clinical samples. It consists of five subscales, namely emotional, physical and sexual abuse and emotional and physical neglect. We specifically used the childhood emotional abuse subscale (CEA, scores may range from 5 to 25), which next to emotional neglect seems to be a stronger predictor of symptoms or diagnosis of depression as compared to physical or sexual abuse (Chapman et al., 2004). In the complete sample internal consistency for the CEA was a= 0.80.

Genotyping

Saliva samples were collected using the Oragene DNA Self-Collection Kit (DISC format; DNA Genotek Inc, Ottawa ON, Canada). Saliva (200 μl) was collected in lysisbuffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/ml proteinase K and 0.5 % w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). Genotyping was performed with a SNPlex assay (Applied Biosystems) and was successful for 96.8% of the samples (genotyping failed for 5 of the initial 155 DNA samples). Genotypes were assessed 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 (**Table 1**, see also **Chapter 3**). The SNPs rs2070950_GC and rs5525_CT were determined as internal controls, as they are in 100% linkage disequilibrium (LD) with the rs2070951 and rs5522 SNPs, respectively (DeRijk et al., 2011). All primer sequences are available upon request.

Statistical analysis

SNP allele frequencies 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). A dichotomous variable was used for sex (1= men; 2= women). A median split variable was created for CEA. Whether subjects had experienced a depressive episode, as diagnosed with the MDQ, was coded as 0= no depression diagnosis, 1= past depression, 2= current depression. Continuous variables were used for age, the LEIDS-R, the HADS and the neuroticism scale.

Differences between men and women on the various psychological factors were tested using an independent-samples *t*-test, a χ^2-test or a Mann-Whitney *U*-test, where appropriate. A square-root transformation was performed for the *Hopelessness/Suicidality*, *Acceptance/Coping*, *Aggression*, *Perfectionism/Control*, HADS-depression and HADS-total scales and a log-transformation was performed for age in order to normalize their distributions (when comparing men and women). After transformation the distribution was still not normal for *Acceptance/Coping*. The Figures 1 and 2 and Tables 1 and 2 represent untransformed data, while statistical tests were performed on transformed data where appropriate (indicated with an asterisk). Table 4 represents transformed data and statistical analysis where appropriate.

To verify whether any of the two functional *MR* SNPs was associated with LEIDS-R, HADS or neuroticism scores, a one-way ANOVA test for a linear trend or an independent-samples *t*-test (for the I180V SNP; because of the low frequencies, subjects carrying the AG or GG genotype were pooled) was used. 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. With multiple linear regression analysis the mean effect of one haplotype 2- or 3-allele on the outcome variable was determined relative to the reference group (haplotype 1 carriers or the subjects carrying no haplotype 2 or 3). To determine the effect of two haplotype 2- or 3-alleles, the effect calculated for one allele can be multiplied by 2. Analyses were repeated while correcting for potential confounding effects of sex (in the total group), age and childhood emotional abuse (CEA; median split: 0= score≤ 6; 1= score> 6). Statistical interaction between the *MR* haplotypes and CEA or sex was verified by adding the two appropriate interaction terms to the model. Because of the sex-specific association between *MR* haplotype 2 and heightened dispositional optimism in the previous study, regression analyses were repeated in both sexes. In addition, association between the haplotypes and neuroticism and current symptoms of anxiety and depression was also tested. Logistic regression was used to test association with self-reported diagnosis of depression. In sensitivity analyses, subjects without a European ancestry were excluded. Finally, regression analysis was repeated while excluding cases with a current depression.

A two-sided *p*-value < .05 was considered statistically significant. Our main interest was the one test determining association between the *MR* haplotypes and hopelessness, specifically in the women. Statistical analysis was performed in SPSS, version 16.0 for Mac OSX (SPSS Inc., Chicago, IL, USA).

Results

Sample characteristics

Characteristics of the 150 students are presented in **Table 1**. LEIDS-R scores were low. Men and women differed on multiple variables; women were significantly younger and scored higher on several subscales of the LEIDS-R and HADS and on neuroticism. Multiple linear regression analysis showed that in women, but not in men, higher hopelessness scores were associated with higher CEA (p= .02).

Table 1 Subject characteristics and *MR* SNP and haplotype frequencies according to sex in 150 students

Variable	Total group n= 150	Women n= 106 (70.7%)	Men n= 44 (29.3%)	*p*-value
Age, mean yrs (SD) *	23.9 (5.0)	23.3 (4.6)	25.4 (5.7)	**.02**
LEIDS-R, mean (SD)				
Hopelessness/Suicidality *	4.9 (4.2)	5.5 (4.3)	3.4 (3.5)	**.002**
Acceptance/Coping #	1.9 (2.3)	1.8 (2.3)	1.9 (2.2)	.91
Aggression *	6.2 (4.3)	6.6 (4.4)	5.3 (4.1)	**.05**
Perfectionism/Control *	7.1 (4.1)	7.7 (3.9)	5.5 (3.9)	**.001**
Risk Aversion	8.5 (4.3)	8.9 (4.2)	7.6 (4.4)	.08
Rumination	10.6 (4.5)	11.1 (4.3)	9.4 (4.9)	**.04**
Total LEIDS-R	39.2 (17.3)	41.7 (16.9)	33.1 (17.0)	**.005**
Symptoms of anxiety, depression				
HADS-anxiety, mean (SD)	6.2 (3.7)	6.92 (3.7)	4.57 (3.0)	**< .001**
HADS-depression, mean (SD) *	2.9 (3.2)	3.22 (3.5)	2.07 (2.0)	**.05**
Total HADS, mean (SD) *	9.1 (6.2)	10.14 (6.5)	6.64 (4.6)	**.001**
Never depressed, %	59.3	54.7	70.5	.18
Past depression, %	32	34.9	25	
Current depression, %	8.7	10.4	4.5	
Neuroticism, mean (SD)	34.3 (9.4)	36.9 (8.7)	27.9 (7.7)	**< .001**
CEA, score> 6, %	49.3	53.8	38.6	.09
MR variants				
-2G/C, GG/CG/CC, freq.	.27 / .45 / .27	.29 / .47 / .24	.23 / .51 / .36	.27
I180V, AA/GA/GG, freq.	.76 / .23 / .01	.74 / .24 / .02	.80 / .20 / .00	.59
MR hap 1 -2G/180A, freq.	.50	.53	.43	.10
MR hap 2 -2C/180A, freq.	.37	.33	.47	
MR hap 3 -2C/180G, freq.	.13	.14	.10	

Notes: Significant *p*-values are indicated in bold. * Statistical test based on transformed data. # Mann-Whitney *U*-test.
Abbreviations: LEIDS-R, Leiden Index of Depression Sensitivity-Revised; HADS, Hospital Anxiety Depression Scale; CEA, childhood emotional abuse.

MR SNP and haplotype frequencies

Allele frequencies of the *MR* SNPs were in HWE (p> .30). Genotype and haplotype frequencies (**Table 1**) and inter-marker correlations between the *MR* -2G/C (rs2070951) and

I180V (rs5522) SNPs (*D'*= 1.0; r^2= .15) were similar as previously found (see **Chapter 4**). There were no individuals carrying a haplotype consisting of the G-allele of the -2G/C SNP combined with the G-allele of the I180V haplotype, this haplotype is very rare. Genotypes for the SNPs rs2070950 and rs5525 were in 100% concordance with the rs2070951 and rs5522 SNPs, respectively. For one of the subjects the genotype for the -2G/C SNP was missing and inferred by the genotype for the rs2070950 SNP. All haplotype probabilities were 1.0.

Associations between the single *MR* SNPs and LEIDS-R scores

The C-allele of the *MR* -2G/C SNP was significantly associated with lower scores for hopelessness, in women but not in men (**Table 2**). In addition, the -2 C-allele associated with lower scores for aggression, risk aversion, rumination and neuroticism. Logistic regression analysis indicated that the *MR* -2G/C SNP associated with self-reported diagnosis of depression according to a dominant model (**Table 3**; the linear model was also significant but showed smaller effect sizes). In men the -2 C-allele seemed to be associated with lower anxiety scores. The *MR* I180V SNP was not significantly associated with differential LEIDS-R scores or scores for any of the other questionnaires, although the number of subjects was too low to appropriately test this. Results were similar after adjustment for potential confounding effects of age and CEA (data not shown).

Table 2 Association between the functional *MR* -2G/C and I180V SNPs and LEIDS-R, HADS and neuroticism scores

			-2G/C (rs2070951)			I180V (rs5522)		
			GG	GC	CC	AA	AG	GG
Hopelessness/Suicidality *	**Total**	mean (SD)	5.10 (4.41)	5.68 (4.27)	3.37 (3.29)	4.82 (4.17)	5.21 (4.24)	3.00 (1.41)
		ANOVA/*t*-test	*F* (1, 147)= 4.09; ***p*= .05**			*t* (146)= -0.14; *p*= .89		
	Women	mean (SD)	6.03 (4.47)	5.90 (4.22)	4.00 (3.84)	5.47 (4.37)	5.76 (4.06)	3.00 (1.41)
		ANOVA/*t*-test	*F* (1, 103)= 3.90; ***p*= .05**			*t* (102)= -0.19; *p*= .85		
	Men	mean (SD)	2.20 (2.78)	5.06 (4.44)	2.38 (1.89)	3.37 (3.30)	3.37 (4.58)	
		ANOVA/*t*-test	*F* (1, 41)= 0.01; *p*= .93			*t* (42)= 0.29; *p*= .78		
Acceptance/Coping *	**Total**	mean (SD)	1.66 (1.94)	1.85 (2.46)	2.02 (2.37)	1.68 (2.23)	2.44 (2.48)	1.00 (1.41)
		ANOVA/*t*-test	*F* (1, 147)= 0.23; *p*= .63			*t* (146)= -1.89; *p*= .06		
	Women	mean (SD)	1.68 (2.12)	1.78 (2.38)	2.16 (2.53)	1.65 (2.20)	2.52 (2.69)	1.00 (1.41)
		ANOVA/*t*-test	*F* (1, 103)= 0.52; *p*= .47			*t* (102)= -1.62; *p*= .11		
	Men	mean (SD)	1.60 (1.35)	2.06 (2.71)	1.81 (2.17)	1.77 (2.33)	2.22 (1.86)	
		ANOVA/*t*-test	*F* (1, 41)= 0.03; *p*= .86			*t* (42)= -0.94; *p*= .35		
Aggression *	**Total**	mean (SD)	7.44 (4.83)	6.07 (3.69)	5.29 (4.64)	6.08 (4.26)	7.03 (4.58)	1.50 (0.71)
		ANOVA/*t*-test	*F* (1, 147)= 3.85; ***p*= .05**			*t* (146)= -1.31; *p*= .19		
	Women	mean (SD)	8.03 (4.70)	6.32 (3.68)	5.44 (4.98)	6.39 (4.26)	7.72 (4.66)	1.50 (0.71)
		ANOVA/*t*-test	*F* (1, 103)= 5.45; ***p*= .02**			*t* (102)= -1.41; *p*= .16		
	Men	mean (SD)	5.60 (5.02)	5.39 (3.74)	5.06 (4.20)	5.37 (4.22)	5.11 (3.95)	
		ANOVA/*t*-test	*F* (1, 41)= 0.02; *p*= .89			*t* (42)= -0.17; *p*= .87		
Perfectionism/Control *	**Total**	mean (SD)	7.98 (4.43)	6.87 (3.87)	6.44 (3.89)	7.16 (4.04)	6.82 (4.22)	5.00 (1.41)
		ANOVA/*t*-test	*F* (1, 147)= 2.45; *p*= .12			*t* (146)= 0.40; *p*= .69		
	Women	mean (SD)	8.19 (4.12)	7.66 (3.91)	7.20 (3.88)	7.73 (3.88)	7.84 (4.28)	5.00 (1.41)
		ANOVA/*t*-test	*F* (1, 103)= 0.68; *p*= .41			*t* (102)= -0.10; *p*= .92		
	Men	mean (SD)	7.30 (5.48)	4.67 (2.85)	5.25 (3.70)	5.86 (4.15)	4.00 (2.50)	
		ANOVA/*t*-test	*F* (1, 41)= 0.84; *p*= .36			*t* (42)= 1.14; *p*= .26		
Risk Aversion	**Total**	mean (SD)	9.59 (4.46)	8.65 (4.20)	7.22 (4.12)	8.71 (4.25)	8.00 (4.63)	6.00 (1.41)
		ANOVA/*t*-test	*F* (1, 147)= 6.35; ***p*= .01**			*t* (146)= 0.84; *p*= .40		
	Women	mean (SD)	10.19 (4.61)	8.62 (3.85)	7.88 (4.28)	9.01 (4.14)	8.80 (4.69)	6.00 (1.41)
		ANOVA/*t*-test	*F* (1, 103)= 4.41; ***p*= .04**			*t* (102)= 0.22; *p*= .83		
	Men	mean (SD)	7.70 (3.53)	8.72 (5.17)	6.19 (3.76)	8.03 (4.46)	5.78 (3.87)	
		ANOVA/*t*-test	*F* (1, 41)= 1.15; *p*= .29			*t* (42)= 1.38; *p*= .17		
Rumination	**Total**	mean (SD)	11.93 (5.13)	10.99 (4.37)	8.73 (3.38)	10.68 (4.76)	10.56 (3.59)	8.50 (4.95)
		ANOVA/*t*-test	*F* (1, 147)= 11.02; ***p*= .001**			*t* (146)= 0.14; *p*= .89		
	Women	mean (SD)	12.97 (4.85)	10.82 (3.86)	9.44 (3.44)	11.19 (4.57)	11.12 (3.11)	8.50 (4.95)
		ANOVA/*t*-test	*F* (1, 103)= 10.60; ***p*= .002**			*t* (102)= 0.07; *p*= .94		
	Men	mean (SD)	8.70 (4.81)	11.44 (5.67)	7.62 (3.07)	9.54 (5.04)	9.00 (4.50)	
		ANOVA/*t*-test	*F* (1, 41)= 0.85; *p*= .36			*t* (42)= 0.29; *p*= .77		
Total LEIDS-R	**Total**	mean (SD)	43.69 (19.28)	40.10 (16.30)	33.07 (15.45)	39.14 (17.71)	40.06 (16.27)	25.00 (7.07)
		ANOVA/*t*-test	*F* (1, 147)= 8.04; ***p*= .005**			*t* (146)= -0.27; *p*= .79		
	Women	mean (SD)	47.10 (17.91)	41.10 (15.54)	36.12 (16.73)	41.45 (17.44)	43.76 (15.03)	25.00 (7.07)
		ANOVA/*t*-test	*F* (1, 103)= 6.20; ***p*= .01**			*t* (102)= -0.60; *p*= .94		
	Men	mean (SD)	33.10 (20.46)	37.33 (18.45)	28.31 (12.20)	33.94 (17.43)	29.78 (15.90)	
		ANOVA/*t*-test	*F* (1, 41)= 0.81; *p*= .37			*t* (42)= 0.65; *p*= .52		
HADS-anxiety	**Total**	mean (SD)	7.24 (3.85)	6.12 (3.64)	5.41 (3.49)	6.31 (3.90)	6.12 (2.99)	4.00 (2.83)
		ANOVA/*t*-test	*F* (1, 147)= 5.13; ***p*= .03**			*t* (146)= 0.26; *p*= .80		
	Women	mean (SD)	7.71 (3.84)	6.56 (3.72)	6.68 (3.66)	7.01 (4.01)	6.88 (2.79)	4.00 (2.83)
		ANOVA/*t*-test	*F* (1, 103)= 1.19; *p*= .28			*t* (102)= 0.15; *p*= .88		
	Men	mean (SD)	5.80 (3.68)	4.89 (3.20)	3.44 (2.06)	4.71 (3.16)	4.00 (2.60)	
		ANOVA/*t*-test	*F* (1, 41)= 4.19; ***p*= .05**			*t* (42)= 0.63; *p*= .54		
HADS-depression *	**Total**	mean (SD)	3.51 (3.41)	2.87 (3.30)	2.27 (2.55)	3.05 (3.38)	2.41 (2.31)	1.00 (0.00)
		ANOVA/*t*-test	*F* (1, 147)= 2.51; *p*= .12			*t* (146)= 0.52; *p*= .60		
	Women	mean (SD)	4.16 (3.59)	2.96 (3.59)	2.56 (2.89)	3.51 (3.74)	2.48 (2.37)	1.00 (0.00)
		ANOVA/*t*-test	*F* (1, 103)= 3.24; *p*= .08			*t* (102)= 0.77; *p*= .44		
	Men	mean (SD)	1.50 (1.65)	2.61 (2.38)	1.81 (1.91)	2.03 (2.05)	2.22 (2.28)	
		ANOVA/*t*-test	*F* (1, 41)= 0.11; *p*= .74			*t* (42)= -0.30; *p*= .77		
Total HADS *	**Total**	mean (SD)	10.76 (6.44)	8.99 (6.21)	7.68 (5.55)	9.36 (6.62)	8.53 (4.47)	5.00 (2.83)
		ANOVA/*t*-test	*F* (1, 147)= 5.71; ***p*= .02**			*t* (146)= 0.19; *p*= .85		
	Women	mean (SD)	11.87 (6.48)	9.52 (6.52)	9.24 (6.10)	10.52 (7.00)	9.36 (4.39)	5.00 (2.83)
		ANOVA/*t*-test	*F* (1, 103)= 2.90; *p*= .09			*t* (102)= 0.24; *p*= .81		
	Men	mean (SD)	7.30 (5.19)	7.50 (5.10)	5.25 (3.51)	6.74 (4.80)	6.22 (4.06)	
		ANOVA/*t*-test	*F* (1, 41)= 1.42; *p*= .24			*t* (42)= 0.17; *p*= .87		
Neuroticism	**Total**	mean (SD)	36.10 (9.45)	35.15 (8.91)	30.93 (9.41)	34.32 (9.60)	34.09 (8.94)	33.50 (4.95)
		ANOVA/*t*-test	*F* (1, 147)= 6.48; ***p*= .01**			*t* (146)= 0.12; *p*= .90		
	Women	mean (SD)	38.94 (8.64)	36.90 (8.33)	34.32 (9.31)	37.05 (9.15)	36.64 (7.72)	33.50 (4.95)
		ANOVA/*t*-test	*F* (1, 103)= 3.91; ***p*= .05**			*t* (102)= 0.20; *p*= .84		
	Men	mean (SD)	27.30 (5.85)	30.28 (8.88)	25.63 (6.93)	28.14 (7.59)	27.00 (8.59)	
		ANOVA/*t*-test	*F* (1, 41)= 0.64; *p*= .43			*t* (42)= 0.39; *p*= .70		

Notes: Data are presented for the total group, as well as for women and men separately. One-way ANOVA *F*-values for linear trend or *t*-statistics (for the I180V SNP) and their accompanying degrees of freedom were used to examine *p*-values for association. Significant *p*-values are indicated in bold. * Statistical test based on transformed data.
Abbreviations: LEIDS-R, Leiden Index of Depression Sensitivity-Revised; HADS, Hospital Anxiety Depression Scale.

Table 3 Results of logistic regression analysis associating the functional *MR* -2G/C and I180V SNPs with self-reported depression 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	-0.71	0.37	.06	0.49 (0.24-1.02)	-0.16	0.36	.67	0.86 (0.42-1.74)
Women	-0.98	0.44	**.03**	0.38 (0.16-0.89)	-0.48	0.42	.26	0.62 (0.27-1.41)
Men	0.15	0.78	.85	1.16 (0.25-5.30)	0.84	0.77	.28	2.31 (0.51-10.54)

Notes: Data are presented for the total group, as well as for women and men separately. Significant *p*-values are indicated in bold. The -2G/C SNP associated with self-reported depression diagnosis according to a dominant model. No clear (linear, dominant, or recessive) relationship was found for the I180V SNP, but because of the low numbers of subjects carrying the AG or GG genotype these genotypes were pooled.

Associations between *MR* haplotypes and LEIDS-R scores

The main aim of this study was to test the association between the three most common *MR* haplotypes and thoughts of hopelessness when in a sad mood, specifically in the women. Indeed, haplotype 2 was significantly associated with fewer thoughts of hopelessness, only among female but not among male students (see **Table 4** and **Figure 1A**). Results were similar after adjustment for age and CEA and the explained variance was 4% (R^2*change*= .04).

Additional analysis of the other five LEIDS-R subscales showed that in women haplotype 2 was also associated with lower scores for aggression (**Figure 1C**), risk aversion (**Figure 1E**) and importantly, rumination (**Figure 1F**, $p < .001$; after a Bonferroni correction for in total twelve tests for the association with six subscales in both sexes, with a significance threshold of $p < .004$, this is still significant). Moreover, in women *MR* haplotype 2 was associated with lower neuroticism scores (**Figure 2D**), a lower odds ratio for self-reported diagnosis of depression (**Table 5**) and a trend was found for less symptoms of depression (**Figure 2B**). **Figures 3** and **4** visualize the number of subjects reporting a diagnosis of depression according to respectively the *MR* haplotypes or diplotypes. **Figure 3** shows that haplotype 2 was related to a lower number of women reporting a diagnosis of depression according to a dominant model (the linear model was also significant but showed smaller effect sizes). This is also clear in **Figure 4**. Unexpectedly, the number of women reporting a diagnosis of depression was also low for the 1/3 diplotype.

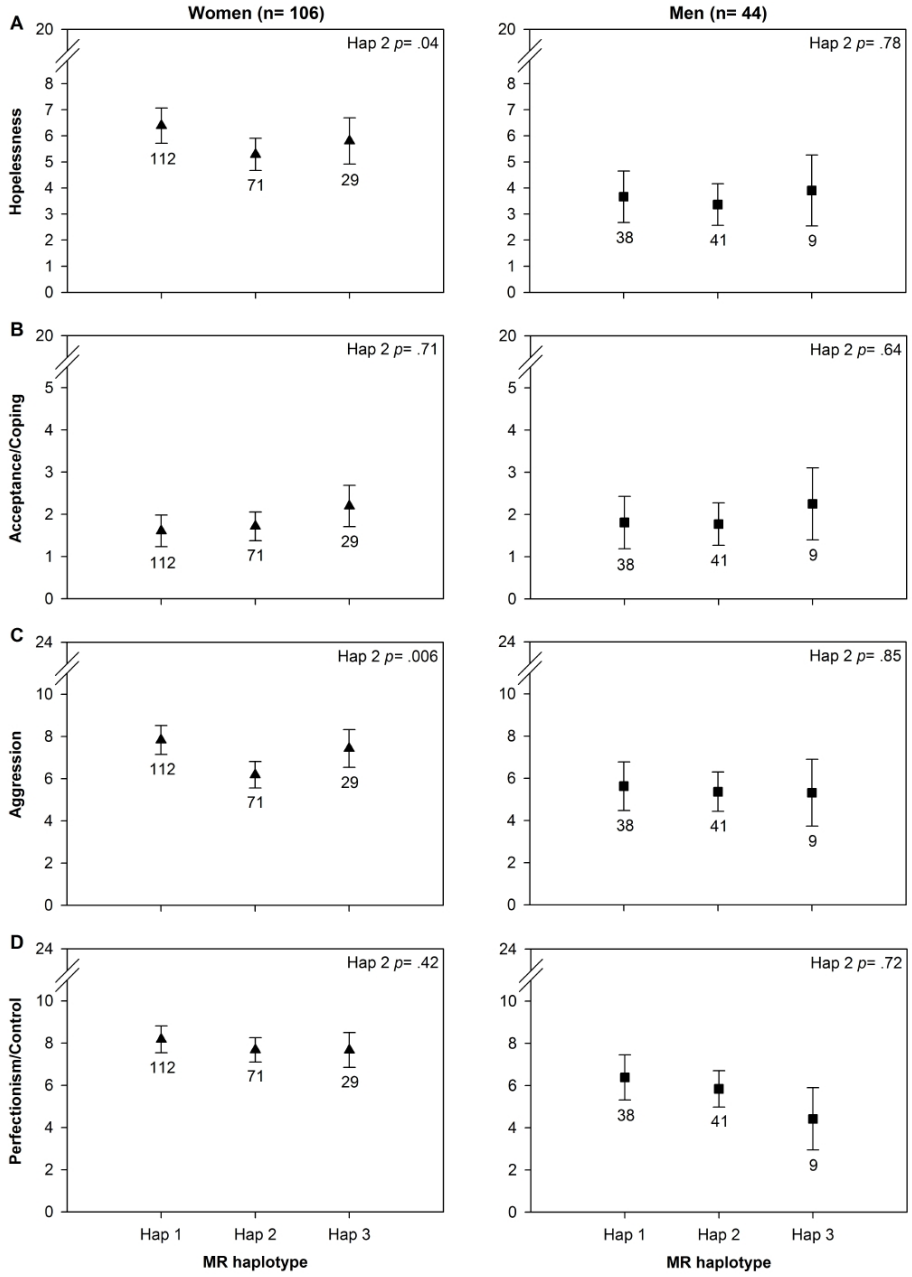
No significant interaction effect was found between the *MR* haplotypes and emotional abuse, although, CEA levels were low (mean (± SD) CEA score in women 8.06 ± 3.41, in men 7.20 ± 3.37, on a range of 5 to 25). Results were similar after correcting for childhood emotional neglect instead of emotional abuse. Finally, results slightly strengthened after excluding subjects (22 women, 4 men) who indicated that one or both of their parents did not have a European ancestry or who did not respond to this question, while excluding subjects reporting the presence of a current depression (11 women, 2 men) gave similar results.

Table 4 Results of linear regression analysis associating three 5' *MR* haplotypes with LEIDS-R, HADS and neuroticism scores, standardized regression coefficients (*b*), *p*-values and R^2 *change*

			MR Hap 2		*MR* Hap 3		
			β	*p*	*β*	*p*	R^2 *change*
Hopelessness/Suicidality *	**Total**	unadjusted	-.18	**.03**	-.05	.55	.03
		adjusted	-.14	.11	-.08	.34	.02
	Women	unadjusted	-.21	**.04**	-.08	.45	.04
		adjusted	-.21	**.04**	-.07	.48	.04
	Men	unadjusted	-.01	.97	-.04	.78	.00
		adjusted	-.05	.78	-.08	.65	.01
Acceptance/Coping *	**Total**	unadjusted	-.01	.92	.12	.16	.02
		adjusted	.00	.98	.09	.32	.01
	Women	unadjusted	.03	.78	.12	.23	.01
		adjusted	.04	.71	.10	.34	.01
	Men	unadjusted	-.10	.52	.13	.40	.03
		adjusted	-.07	.64	.02	.91	.01
Aggression*	**Total**	unadjusted	-.20	**.02**	-.01	.89	.04
		adjusted	-.18	**.04**	-.02	.79	.03
	Women	unadjusted	-.27	**.008**	-.05	.62	.07
		adjusted	-.28	**.006**	-.04	.73	.07
	Men	unadjusted	.01	.93	.03	.87	.00
		adjusted	.03	.85	.03	.86	.00
Perfectionism/Control *	**Total**	unadjusted	-.12	.15	-.08	.34	.02
		adjusted	-.08	.36	-.09	.31	.01
	Women	unadjusted	-.08	.45	-.06	.59	.01
		adjusted	-.08	.42	-.04	.69	.01
	Men	unadjusted	-.07	.64	-.18	.25	.04
		adjusted	-.06	.72	-.17	.33	.03
Risk aversion	**Total**	unadjusted	-.19	**.03**	-.14	.10	.04
		adjusted	-.17	**.05**	-.15	.08	.04
	Women	unadjusted	-.20	**.05**	-.12	.22	.04
		adjusted	-.21	**.05**	-.12	.26	.04
	Men	unadjusted	-.08	.60	-.22	.16	.05
		adjusted	-.06	.73	-.25	.14	.06
Rumination	**Total**	unadjusted	-.28	**.001**	-.11	.20	.08
		adjusted	-.28	**.001**	-.09	.26	.07
	Women	unadjusted	-.33	**.001**	-.14	.15	.10
		adjusted	-.35	**< .001**	-.09	.34	.11
	Men	unadjusted	-.13	.42	-.06	.70	.02
		adjusted	-.12	.45	-.06	.74	.02
Total LEIDS-R	**Total**	unadjusted	-.25	**.004**	-.08	.31	.06
		adjusted	-.22	**.01**	-.10	.25	.04
	Women	unadjusted	-.26	**.01**	-.09	.35	.06
		adjusted	-.27	**.006**	-.08	.45	.07
	Men	unadjusted	-.10	.51	-.11	.48	.02
		adjusted	-.08	.61	-.13	.46	.02
HADS anxiety	**Total**	unadjusted	-.18	**.03**	-.10	.25	.03
		adjusted	-.15	.07	-.09	.26	.02
	Women	unadjusted	-.09	.37	-.09	.36	.01
		adjusted	-.11	.27	-.05	.62	.01
	Men	unadjusted	-.29	.06	-.13	.39	.09
		adjusted	-.28	.08	-.12	.47	.08
HADS depression *	**Total**	unadjusted	-.12	.15	-.09	.31	.02
		adjusted	-.11	.18	-.08	.31	.02
	Women	unadjusted	-.15	.13	-.15	.16	.03
		adjusted	-.18	.06	-.10	.33	.03
	Men	unadjusted	.03	.83	.05	.76	.00
		adjusted	.06	.73	.01	.96	.00
Total HADS *	**Total**	unadjusted	-.20	**.02**	-.09	.26	.04
		adjusted	-.17	**.04**	-.09	.25	.03
	Women	unadjusted	-.15	.13	-.12	.24	.03
		adjusted	-.18	.06	-.07	.49	.03
	Men	unadjusted	-.19	.22	-.05	.76	.04
		adjusted	-.16	.31	-.08	.64	.03
Neuroticism	**Total**	unadjusted	-.22	**.008**	-.07	.40	.05
		adjusted	-.16	**.04**	-.10	.19	.03
	Women	unadjusted	-.20	**.05**	-.10	.32	.04
		adjusted	-.21	**.03**	-.08	.41	.04
	Men	unadjusted	-.11	.50	-.07	.64	.02
		adjusted	-.06	.70	-.14	.42	.02

Notes: Data are presented for the total group, as well as for women and men separately. Effect per haplotype allele was calculated as compared to the reference group (haplotype 1 carriers/subjects carrying no haplotype 2 or 3). Adjusted results are corrected for sex (in the total group), age and emotional abuse (median split). Significant *p*-values are indicated in bold. * Transformed data.

Abbreviations: LEIDS-R, Leiden Index of Depression Sensitivity-Revised; HADS, Hospital Anxiety Depression Scale.


A
B
C
D
Women (n= 106)
Men (n= 44)
Hopelessness
Acceptance/Coping
Aggression
Perfectionism/Control
Hap 2 p= .04
Hap 2 p= .78
Hap 2 p= .71
Hap 2 p= .64
Hap 2 p= .006
Hap 2 p= .85
Hap 2 p= .42
Hap 2 p= .72
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71
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9
Hap 1
Hap 2
Hap 3
MR haplotype

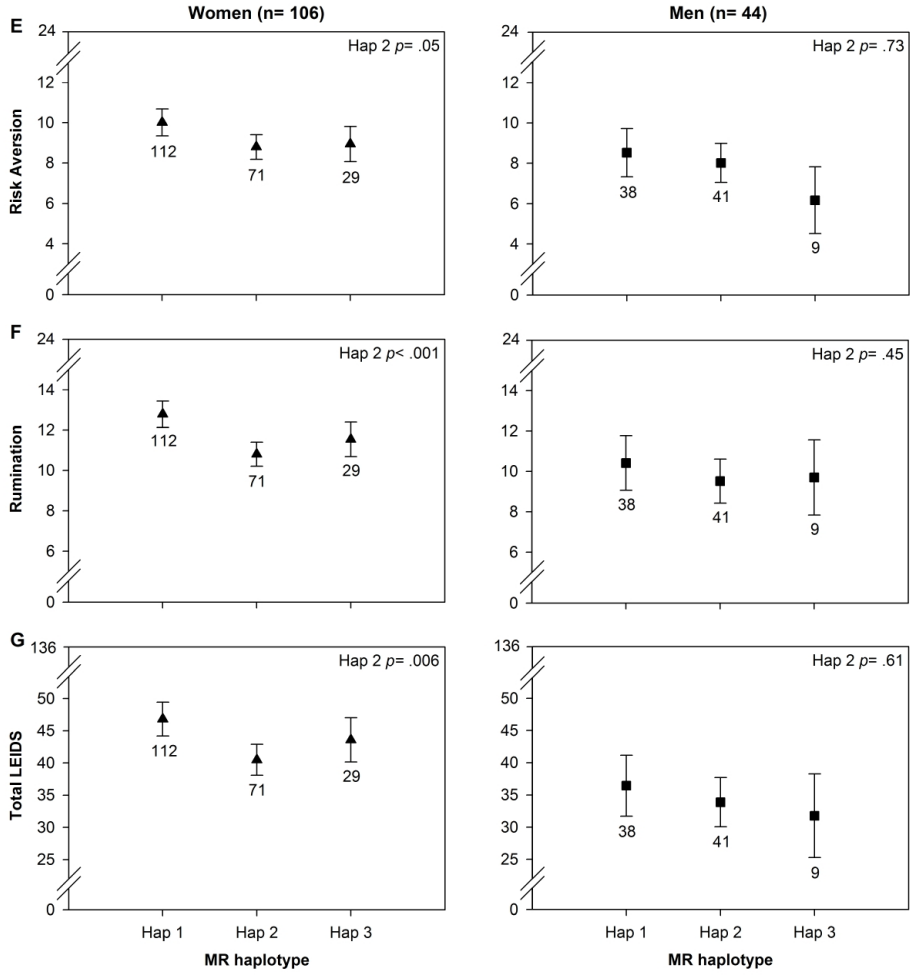

Figure 1 Crude mean scores (± SEM) for the LEIDS-R and its subscales according to three 5' *MR* haplotypes in women and men. The figure shows differences in scores based on one haplotype allele. To determine the effect of two haplotype 2- or 3-alleles, the effect calculated for one allele can be multiplied by 2. *P*-values represent adjusted comparison of haplotype 2 to the reference (haplotype 1 carriers) with linear regression. Note the breaks in the y-axis. * Statistical test based on transformed data.

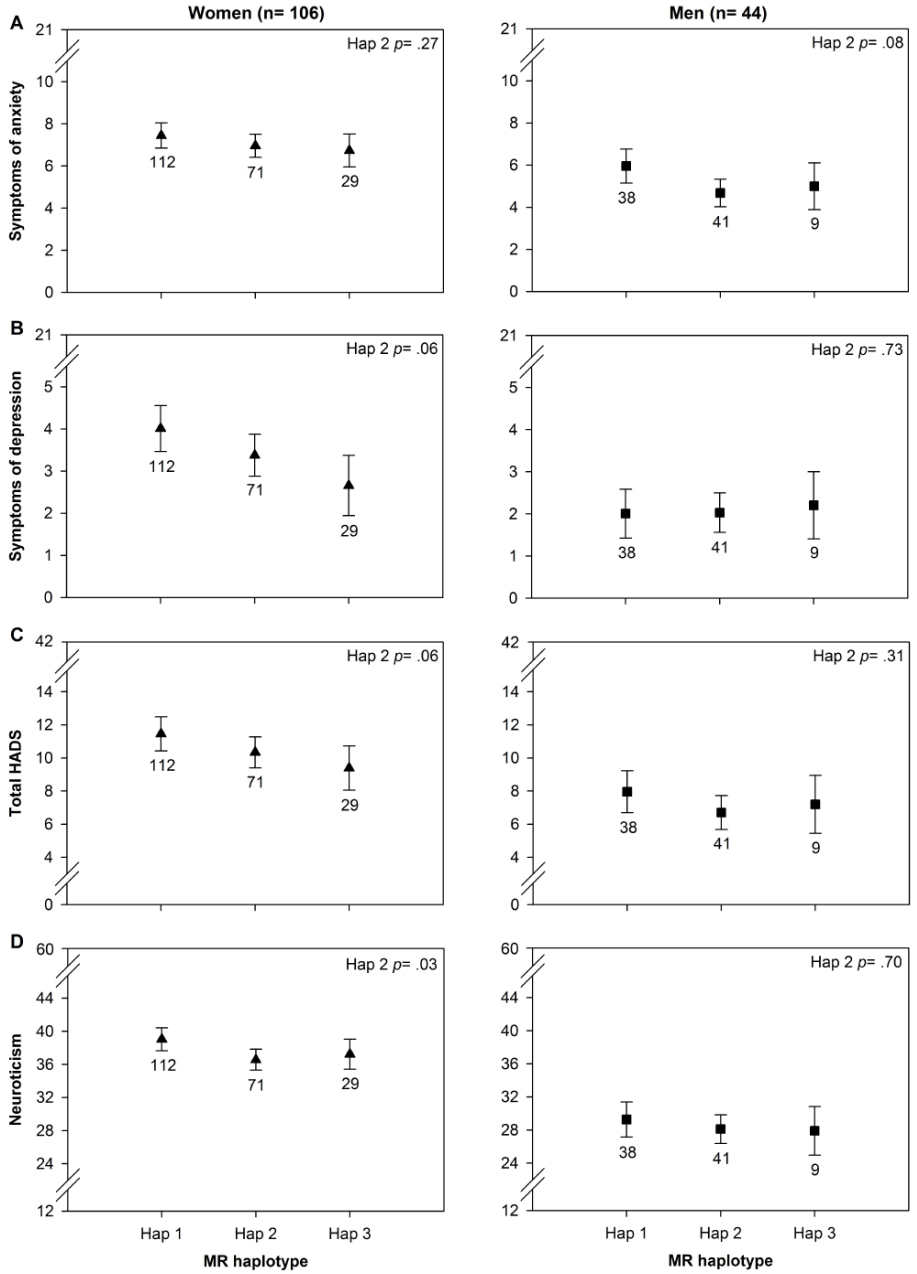

Figure 2 Crude mean scores (± SEM) for the HADS, its subscales and neuroticism according to three 5' *MR* haplotypes in women and men. The figure shows differences in scores based on one haplotype allele. To determine the effect of two haplotype 2- or 3-alleles, the effect calculated for one allele can be multiplied by 2. *P*-values represent adjusted comparison of haplotype 2 to the reference (haplotype 1 carriers) with linear regression. Note the breaks in the y-axis. * Statistical test based on transformed data.

Table 5 Results of logistic regression analysis associating three 5' *MR* haplotypes with self-reported depression 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%*	*B*	*SE*	*p*	*odds ratio (95%*
Total	unadjusted	-0.53	0.35	.13	0.59 (0.30-1.16)	-0.32	0.41	.43	0.73 (0.33-1.61)
	adjusted	-0.71	0.39	.07	0.49 (0.23-1.05)	-0.20	0.45	.66	0.82 (0.34-1.97)
Women	unadjusted	-0.69	0.42	.10	0.50 (0.22-1.14)	-0.83	0.49	.09	0.50 (0.22-1.14)
	adjusted	-1.04	0.48	**.03**	0.36 (0.14-0.91)	-0.59	0.54	.27	0.55 (0.19-1.60)
Men	unadjusted	-0.15	0.73	.83	0.86 (0.20-3.61)	0.85	0.78	.27	2.35 (0.51-10.79)
	adjusted	-0.29	0.77	.70	0.75 (0.16-3.39)	0.88	0.87	.31	2.40 (0.44-13.19)

Notes: Data are presented for the total group, as well as for women and men separately. Haplotype 2 associated with self-reported depression diagnosis according to a dominant model. 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). Adjusted results are corrected for sex (in the total group) age and emotional abuse (median split). Significant *p*-values are indicated in bold.

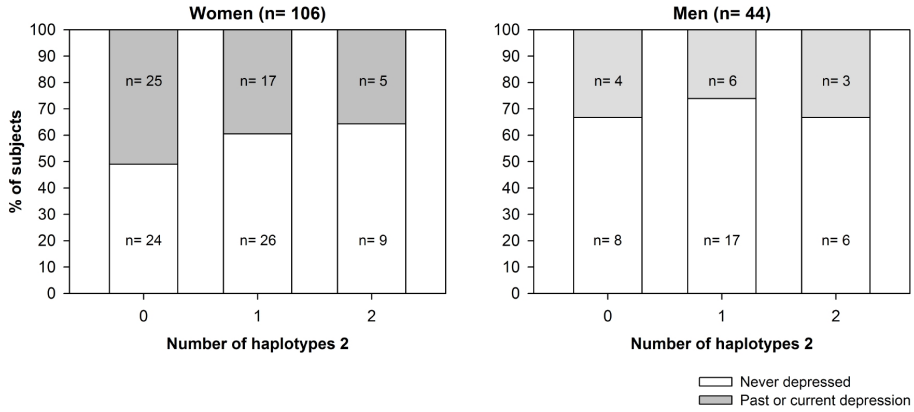

Figure 3 Percentage of students reporting a diagnosis of depression according to the number of haplotypes 2.

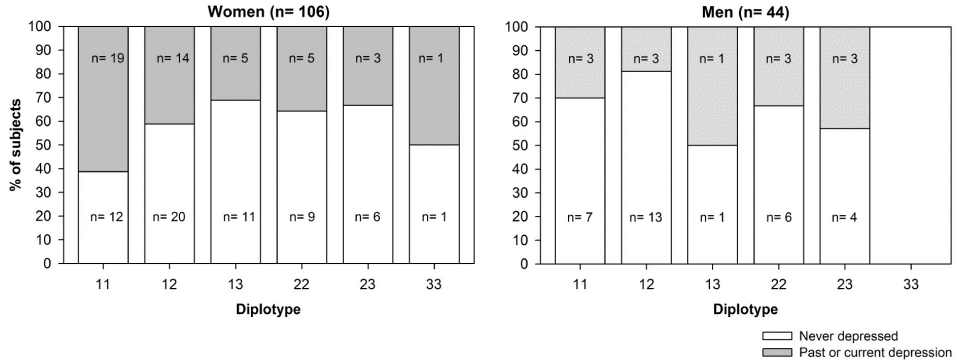

Figure 4 Percentage of students reporting a diagnosis of depression according to the six 5' *MR* diplotypes.

Discussion

We hypothesized that the MR modulates aspects of cognitive functioning that are underlying psychological health. Here we report that a common *MR* haplotype, including the -2 C-allele and the 180 I-allele, associated with less thoughts of hopelessness during sadness. In addition, this haplotype also associated with less aggression, less risk aversion and, importantly, less rumination. Moreover, haplotype 2 also associated with less neuroticism and a lower odds ratio for depression. Associations were found in female but not in male students. These results are in line with our previous study showing that this same haplotype associated with heightened levels of dispositional optimism among female elderly (**Chapter 4**). Although cognitive reactivity seems to depend highly on childhood trauma, in part through interaction with differential susceptibility genes (Antypa and Van der Does, 2010b), the effect of *MR* haplotype 2 on cognitive reactivity was independent of childhood trauma.

As already mentioned the *MR* genetic variants described here significantly affect MR activity. Experimental studies in cell lines showed that haplotype 2 (-2C/180I) results in higher MR protein expression and higher transactivation of target genes after binding its ligand cortisol (van Leeuwen et al., 2011). The -2G/C and I180V SNPs are linked to multiple SNPs in the *MR* gene promoter region. These SNPs were found to confer higher *MR* transcription in cells under non-stimulated conditions (**Chapter 3**). Several of these SNPs were predicted to influence transcription factor binding, which could result in differential MR expression in a context-dependent manner. It is likely that these functional SNPs are at least in part responsible for the variability in neuroendocrine and behavioral regulation. Indeed, in humans the -2G/C and I180V SNPs and their related haplotypes associate with variability in neuroendocrine activity, particularly becoming clear under the influence of a 'challenge' (e.g. dexamethasone treatment, exposure to a psychosocial stressor, use of selective serotonin reuptake inhibitors) (DeRijk et al., 2006; Kuningas et al., 2007; van Leeuwen et al., 2010a; van Leeuwen et al., 2011) (Chapter 6). In addition, the functional *MR* genetic variants seem to modulate psychological wellbeing. While among elderly the 180 V-allele has been shown to relate to more feelings of depression (Kuningas et al., 2007), a more recent study

reported on *MR* haplotype-related variability in perceived chronic stress at work among teachers; haplotype 2 carriers experienced less stress (van Leeuwen et al., 2011).

Again, the associations found depended on gender. Literature on sex-differences in brain functioning is increasing, implicating sex hormones and sex-dependent signaling pathways (Cahill, 2006; Jazin and Cahill, 2010). With respect to the MR it is important to know that the *MR* haplotypes establish differences in MR activity with varying ligand availability *in vitro* (van Leeuwen et al., 2011), while HPA responses to stress are gender-specific (Kudielka and Kirschbaum, 2005). Moreover, estrogens, androgens and progesterone 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). Possibly these sex steroids modulate MR expression at the level of the promoter region. *In vitro* studies are needed to verify this hypothesis.

The present data add to the hypothesis that differences in central MR signaling may underlie in part the development of depression (Holsboer, 2000). People at risk of depression are thought to cope less efficient with challenges at a neuroendocrine and behavioral level (Southwick et al., 2005). Multiple lines of evidence indicate that cortisol modulates appraisal and cognitive flexibility through the MR when dealing with a challenge (Oitzl et al., 1994; Berger et al., 2006; Otte et al., 2007; Bogdan et al., 2010). The present data indicate that the MR also modulates psychological resilience. Hopelessness, rumination and neuroticism, but also dispositional optimism (**Chapter 4**), are traits that potentially predict the risk of depression, whereas hopelessness is also related to suicidal ideation during and between depressive episodes (Nolen-Hoeksema, 2000; Giltay et al., 2006a; Kendler et al., 2006; Lakdawalla et al., 2007; Antypa et al., 2010a).

The mechanism through which glucocorticoids and the MR relate to the traits described here remains unclear. Supportive literature exists on a role for cortisol and its receptors in traits like hopelessness and rumination (Jacobs et al., 1997; Zoccola et al., 2008). In addition, animal studies indicate that the MR plays a role in learned helplessness, which is highly linked to hopelessness. Specifically when corticosterone levels were moderate, a situation where only the MR is highly occupied due to its high affinity (Reul and de Kloet, 1985), animals show lower learned helplessness compared to high corticosterone levels, a situation where in addition to the MR also the GR becomes occupied (Kademian et al., 2005). Yet, recent studies have identified a membrane-bound MR, which mediates fast responses to stress-induced levels of cortisol (Karst et al., 2005; Joels et al., 2008; Karst et al., 2010). Hopelessness and optimism may be linked to the system for reward and motivation (Southwick et al., 2005). Indeed, glucocorticoids are known to act on the brain reward system (de Jong and de Kloet, 2004; Fiancette et al., 2010). The results of a recent genetic association study fit with this idea of a link with the reward system, showing that *MR* 180 V-allele carriers were less able to modulate behavior as a function of reward after an acute stressor (Bogdan et al., 2010).

This study has several limitations. The present results are based on a small sample of only 150 students, including less than half the number of males compared to females. No hard

conclusions can therefore be drawn particularly based on the results for the *MR* I180V SNP and haplotype 3. In addition, cognitive reactivity, neuroticism and the presence of anxiety and depression were assessed with self-report questionnaires and not with an interview, which could slightly obscure the results. Moreover the presence of other physiological or psychiatric disorders was not excluded. Finally, no data were available on the use of antidepressants or oral contraceptives, while it is known that antidepressants or sex steroids can influence corticosteroid receptor expression or even interact with the *MR* SNPs in their effect on neuroendocrine regulation (**Chapter 6**). Still, the results perfectly fit with the previous finding of an association between haplotype 2 and increased dispositional optimism in women (**Chapter 4**).

The current data suggest that the *MR* genotype is an important factor implicated in inter-individual differences in psychological resilience to stress-related psychopathology. While in a previous study among elderly haplotype 2 associated with heightened levels of dispositional optimism, in the present study among students haplotype 2 associated with less thought of hopelessness and less rumination during sadness. These associations were found only in women, making specifically them less vulnerable for future depression. Indeed, *MR* haplotype 2 associated with a lower odds ratio for self-reported diagnosis of depression according to a dominant model. Again, these results have to be interpreted with caution, as these data are based on a self-report of depression diagnosis and not on a clinical interview and the number of subjects was low. Therefore, the results need to be replicated (see **Chapter 7**). The fact that the associations between *MR* genotype and psychological functioning were only observed in females is of interest in view of the two times higher prevalence of depression in women as compared to men (Bijl et al., 1998). Longitudinal studies examining whether the *MR* haplotype 2 provides resilience to mental disorders are warranted. 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). To conclude, the present study shows that the *MR* gene modulates an individual's cognitive response to sad mood. Future studies should establish whether the MR is a valuable marker for the prediction and a target for the prevention and/or treatment of symptoms of depression.

Acknowledgements

This research was supported by the Netherlands Brain Foundation (Hersenstichting Nederland, 10F02(2).37), the Netherlands Organisation for Scientific Research (NWO, 453-06-005), psychiatric hospital Rivierduinen and the Royal Netherlands Academy of Arts and Sciences. Patrizia Rizzu, PhD is thanked for SNP genotyping.