Supplementary Information for

A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females

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Supplementary Materials and Methods

PCR and sequencing of the *MR* promoter region

Because of the high GC content of the *MR* promoter region a PCR kit specifically designed for GC-rich regions was used, the GC-RICH PCR system (Roche Diagnostics, Mannheim, Germany). PCR-reactions were performed in a total volume of 25 μL and contained 1 x buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Invitrogen Life Technologies, Breda, The Netherlands), 0.3 μM forward and reverse primer (Isogen Life Science, Maarssen, The Netherlands), 0.5 M solution 3, 1 unit of enzyme and 50 ng of DNA. PCR was performed in a PTC-200 (MJ Research Inc, Watertown, MA, USA) using the following conditions: an initial denaturation step for 3 min at 95°C, followed by 35 cycles of 60 sec (or 90 sec for products larger than 1 kb) at 95°C, 30 sec at the specific annealing temperature and 60 sec (or 90 sec for products larger than 1 kb) at 72°C and a final extension step of 7 min at 72°C. The PCR products were purified with columns (Machery-Nagel, Düren, Germany) followed by sequencing PCR. Sequencing PCR was performed with the BigDye Terminatior v3.1 Cycle Sequencing Kit (Applied Biosystems, Niewerkerk a/d IJssel, The Netherlands) according to the manufacturers instructions and products were analyzed on an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems). The 4 kb sequence of the *MR* promoter region was initially analyzed using seven different primer sets (**Supplementary Table 1**). Sequencing PCR was performed using the same forward and/or reverse primer. Nested primers were used where sequences were unclear. In case of detection of a novel SNP (sequence compared to NCBI36:4:149582036:149585904, Ensembl, January 2007) a second set of primers was used to validate.

Prediction of SNP effects on *MR* transcription

Based on the literature we verified whether any of the common *MR* 5' UTR SNPs were located in previously identified transcription factor (TF) binding sites.[1] Moreover, common SNPs were analyzed for allele-specific binding of matrix conservation based predicted glucocorticoid responsive elements (GREs) or allele-specific TF binding with the help of the TRANSFAC 4.0 database[2] (using the tool AliBaba 2.1, September 2010, minimal matrix conservation set at 75%, available

online, http://www.gene-regulation.com/pub/programs.html) and the JASPAR database (JASPAR CORE homo sapiens database, September 2010, relative profile score threshold set at 75%, tool available online, http://jaspar.genereg.net).[3] Finally, effects of the SNPs on splicing of the MR transcripts were explored (http://www.fruitfly.org/seq_tools/splice.html).[4]

Construction of reporter plasmids

For the construction of reporter plasmids containing the MR promoter region with haplotype 1, 2, or 3, the sequence of 3870 basepairs was amplified using DNA of a subject that was identified as carrying one of these haplotypes. To the forward primer eight nucleotides were added at the 5' end including six nucleotides encoding a restriction site for the enzyme *Acc65*I, while to the reverse primer eight nucleotides were added at the 3' end including six nucleotides encoding a restriction site for the enzyme *Bgl*II (**Supplementary Table 1**). PCR-reactions contained 1 x buffer (GC-RICH PCR system, Roche), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 μM forward and reverse primer, 0.5 M solution 3, 1 unit of enzyme and 50 ng of DNA in a total volume of 25 μL. PCR was performed using the following conditions: an initial denaturation step for 3 min at 95°C, followed by 35 cycles of 1 min at 95°C, 30 sec at 62°C and 3 min at 68°C and a final extension step of 7 min at 72°C. The PCR products were purified with columns, cloned into the intermediate vector pGEM-T Easy (Promega, Leiden, The Netherlands) and sequenced to confirm the nucleotide sequence. Subsequently, the *MR* promoter sequences were cut out of the pGEM-T Easy vector with the restriction enzymes *Acc65*I and *Bgl*II and ligated (T4 DNA ligase, Promega) into the pGL3-Basic luciferase reporter vector (Promega), which was cut open with the same restriction enzymes.

Cell culture, transient transfection and luciferase assays

Human neuroblastoma cells (BE(2)-M17; Health Protection Agency Culture Collections, Cat. No. 95011816) were kept at 70-80% confluence in GIBCO Opti-MEM I Reduced Serum Medium with L-Glutamine (Invitrogen Life Technologies, Breda, The Netherlands) supplemented with 10% Fetal Bovine Serum (FBS, GIBCO) and 1% penicillin/streptomycin (Pen/Strep, GIBCO) at 37°C in a 5% CO_2 atmosphere. One day before transfection 50.000 cells were seeded in 24-well plates in

growth medium without Pen/Strep. For transfection 1 μL of Lipofectamine 2000 (Invitrogen) was used to transfect 200 ng of the haplotype-firefly luciferase construct 1, 2 or 3, together with 10 ng of pGL4.74[*hRluc*/TK] *Renilla* luciferase reporter vector (Promega). In separate wells, 100 ng of pGL3-Basic or pGL3-Control vector (Promega) were transfected, functioning as respectively background measurement or positive control. Four hours after transfection, culture medium was removed and fresh growth medium with Pen/Strep was applied to the cells. Each construct was transfected in six separate wells. After 48 hours of incubation cells were lysed in 100 μL of Passive Lysis Buffer (PLB, Promega). Firefly and *Renilla* luminescent activity was assessed sequentially in 25 μL of lysate after addition of 25 μL of Luciferase Assay Buffer II followed by addition of 25 μL of Stop & Glo Buffer (Promega). Luminescent signals were measured for 10 sec in a Berthold CentroXS^3 LB 960 Microplate Luminometer (Berthold, Bad Wildbad, Germany).

Study population 1

Of the original sample of 1793 individuals, 1012 subjects gave an interview, 641 subjects gave a blood sample, 499 (77.8%) DNA isolates were available for genotyping, which was successful for 473 (94.8%) DNA samples. The final subset of 450 subjects (optimism scores were missing for 23 subjects) were not statistically significantly different from the initial group of 1012 subjects that gave an interview on sex, education or total number of chronic diseases. The included subjects were, however, significantly younger (mean age 73.7 ± 5.7 vs. 75.2 ± 5.7, $p< 0.001$), more often together (60.9% vs. 54.1%, $p= 0.03$), more often had a higher socioeconomic status (SES; 64.0% vs. 55.8%, $p< 0.01$), more often suffered from cardiovascular disease (CVD; 24.0% vs. 14.9%, $p= 0.01$), and were more optimistic (mean score 13.40 ± 4.68 vs. 12.36 ± 4.91, $p= 0.001$). One subject reported a depressive disorder.

Study population 2

The second study sample consisted of 150 university students (5 subjects of the original group of 155 subjects (3.2%) were excluded because of failed genotyping) that were part of a study testing the relation between the serotonin transporter gene and cognitive vulnerability for depression.[5] The Leiden Index of Depression

Sensitivity-revised (LEIDS-R) was used to assess cognitive reactivity (CR)[6], which next to the *Hopelessness/Suicidality* subscale includes subscales for *Acceptance/Coping* (e.g., "When in a sad mood, I feel more like myself"), *Aggression* ("When I feel down, I lose my temper more easily"), *Perfectionism/ Control* (e.g., "When in a sad mood, I become more bothered by perfectionism"), *Risk Aversion* (e.g., "When in a low mood, I take fewer risks") and *Rumination* (e.g., "When I feel sad, I spend more time thinking about the possible causes of my moods"). 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*.

Neuroticism was assessed using 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).[7] Current symptoms of anxiety and depression were assessed using the 14-item Dutch version of the Hospital Anxiety and Depression Scale (HADS) self-report scale.[8] Presence of current and past depression was assessed with the Major Depression Questionnaire (MDQ), which covers all DSM-IV criteria for current and past depression.[9]

Study population 3

MDD cases (n= 1730) were mainly from the Netherlands Study of Depression and Anxiety (NESDA; http:// www.nesda.nl).[10] 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.[11] 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.[12] 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.[13]

DNA isolation and genotyping

Genomic DNA was isolated from the blood samples of the first study group according

to standard procedures. Genotyping of these samples was performed using a Sequenom MassARRAY iPLEX assay (Sequenom, San Diego, CA, USA), mass differences were detected using an Autoflex (Bruker, Wormer, Netherlands) MALDI-TOF Mass Spectrometer and genotypes were assigned real-time using MassARRAY TYPER Analyzer 3.4 software (Sequenom, San Diego, CA, USA). Genotyping was successful for 94.8% of the samples.

Saliva samples of the second study group were collected and DNA was isolated using the Oragene Self-Collection Kit –DISC format (DNA Genotek Inc, Ottawa ON, Canada). 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. All primer sequences are available upon request. In study group 1 and 2 genotypes for 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 respectively.[14]

For the patients as well as the control subjects of study 3 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.[13]

Supplementary Results

Additional results found in study population 1

Additional analysis of the other four SSWO subscales showed that only in women haplotype 2 was also associated with higher levels of self-respect (change in score of 1.1 per allele; p= 0.002), a statistical trend was found for higher morale (p= 0.07) and better subjective health (p= 0.08) while no relation was found with appreciation of social contacts (p= 0.60).

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Supplementary Table 1 Locations, sequences, annealing temperatures and product sizes of primers used for SNP analysis of 4 kb of the human *MR* promoter region

Primer location	Sequence	Ann. Temp.	Product size
Promoter 2 Promoter 2	FWD: 5'-CGA GGA GCA GGA AAA GAA AA-3' REV: 5'-GGT GAG GAT GGA GAG GAT GA-3'	56	670
Promoter 2 Promoter 1	FWD: 5'-GAT CCT CCT GCC GGA CTT-3' REV: 5'-CCC TGG ATC TCA GCT TCT TG-3'	58	610
Promoter 1 Promoter 1	FWD: 5'-CCG CCT CTT GTA GGG TAA CA-3' REV: 5'-CCC TGG ATC TCA GCT TCT TG-3'	58	452
Promoter 1 Promoter 1 Promoter 1 Promoter 1	FWD: 5'-GAC AGT CAC TTT GCG CTG AC-3' REV: 5'-AAT TTC GGT TTC CCT CCA AC-3' REV: 5'-GGA ACT CCC TGG AGA TAG GG-3' FWD: 5'-GGC ATT AGA GTC TGG GGT CA-3'	56	1010
Promoter 1 Promoter 1	FWD: 5'-GGG GGA CCA GAT TTA GGT GT-3' REV: 5'-CAC CCT GCT CTC CTT CTG AC-3'	58	868
Promoter 1 Promoter 1	*FWD: 5'-GGC ATT AGA GTC TGG GGT CA-3'* *REV: 5'-AAG AAG TGG CAG GGT CAA GA-3'*	57	696
Promoter 1 Intron 1	FWD: 5'-AGA CAG TGG AAA GGG GCT G-3' REV: 5'-TCC TTC AAC TGC CCT TATG C-3'	57	1322
Promoter 1 Promoter 1	*FWD: 5'-AGA CAG TGG AAA GGG GCT G-3'* *REV: 5'-TCT CTC GCC GTC TAC CTG TT-3'*	59	895
Promoter 1 Promoter 1	*FWD: 5'-CAG GGT GGA CGT AAG CAA GT-3'* *REV: 5'-TCT CTC GCC GTC TAC CTG TT-3'*	59	662
Exon1α Exon1α	*FWD: 5'-AAC AGG TAG ACG GCG AGA GA-3'* *REV: 5'-AGG AAG CGT AGC CTG TCT CA-3'*	57	548
Intron 1 Intron 1	FWD: 5'-TAC CAC CCT TCC CTT TAC CC-3' REV: 5'-GGT TTC AAA AGC TCG TCT GC-3'	58	469
Promoter 2 Intron 1	FWD: 5'-CG**G GTA CC**C GAG GAG CAG GAA AAG AAA A-3' REV: 5'-CG**A GAT CT**G GTT TCA AAA GCT CGT CTG C-3'	62	3886

Notes: A 4kb sequence of the human *MR* promoter region was analyzed using initially seven different primer sets. Sequencing PCR was performed using the same forward and/or reverse primer. Additional sequencing primers (underlined) were used in case the sequence was not clear along the complete amplification product. Additional PCR primer sets (italic) were used to verify the results of the first set of primers in case novel SNPs were detected. The last set of primers was used for amplification of the complete 4 kb *MR* promoter region and the product was used for reporter plasmid construction. Sequences for restriction factor binding sites are indicated in bold text. Abbreviations: FWD, forward primer; REV, reverse primer; Ann. Temp., annealing temperature.

Supplementary Table 2 Allele- and genotype frequencies of 16 SNPs located along 4 kb of the human *MR* promoter region

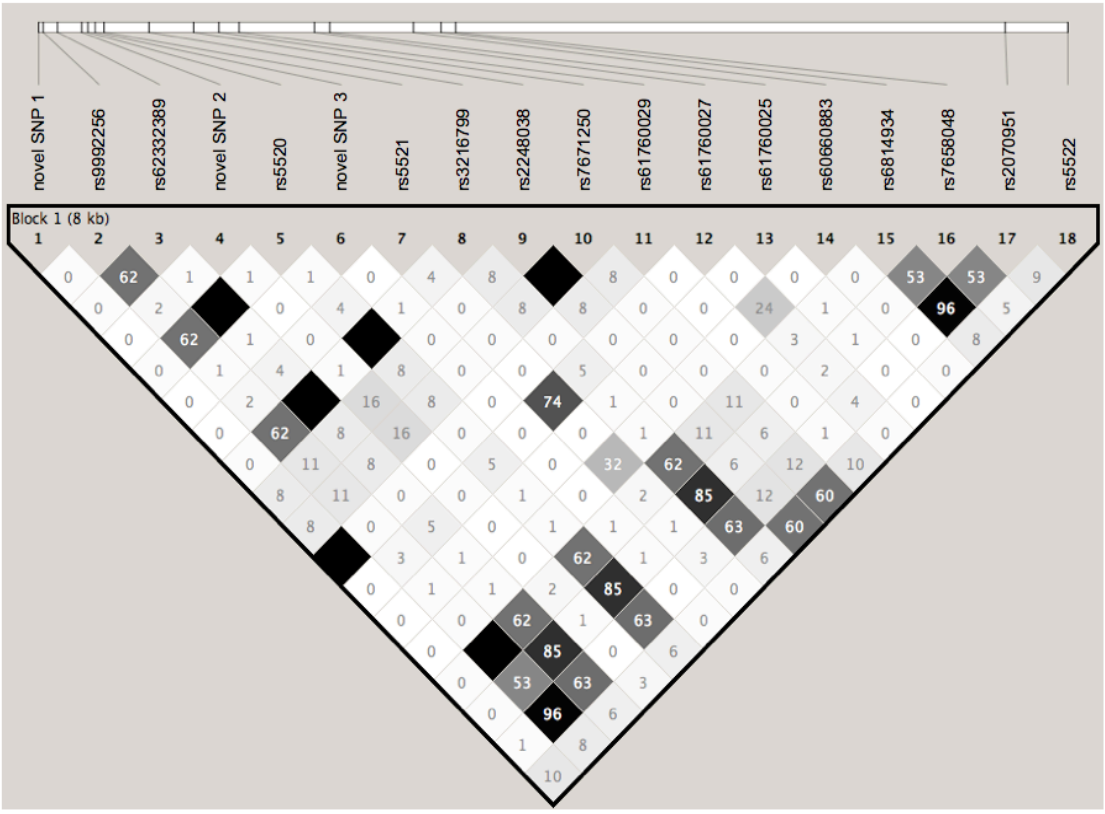
SNP	SNP number	Location	Alleles n / allele / frequency						Genotypes n / genotype / frequency									HWE (*p*)
1	Novel SNP 1 G/C	149366331	103	G	0.99	1	C	0.01	51	G/G	0.98	1	G/C	0.02	0	C/C	0.00	1.00
2	**rs9992256 C/T**	149366293	**54**	**C**	**0.52**	**50**	**T**	**0.48**	**12**	**C/C**	**0.23**	**30**	**C/T**	**0.58**	**10**	**T/T**	**0.01**	**0.44**
3	**rs62332389 C/T**	149366170	**62**	**C**	**0.60**	**42**	**T**	**0.40**	**16**	**C/C**	**0.31**	**30**	**C/T**	**0.58**	**6**	**T/T**	**0.11**	**0.28**
4	Novel SNP 2 G/A	149365962	102	G	0.98	2	A	0.02	50	G/G	0.96	2	G/A	0.04	0	A/A	0.00	1.00
5	**rs5520 G/C**	149365909	**62**	**G**	**0.60**	**42**	**C**	**0.40**	**16**	**G/G**	**0.31**	**30**	**G/C**	**0.58**	**6**	**C/C**	**0.11**	**0.28**
6	Novel SNP 3 C/A	149365846	103	C	0.99	1	A	0.01	51	C/C	0.98	1	C/A	0.02	0	A/A	0.00	1.00
7	rs5521 T/C	149365769	101	T	0.97	3	C	0.03	49	T/T	0.94	3	T/C	0.06	0	C/C	0.00	1.00
8	**rs3216799 -/CT**	**149365384**	**62**	**-**	**0.60**	**42**	**CT**	**0.40**	**16**	**-/-**	**0.31**	**30**	**-/CT**	**0.58**	**6**	**CT/CT**	**0.11**	**0.28**
9	**rs2248038 G/A**	149364999	**11**	**G**	**0.11**	**93**	**A**	**0.89**	**1**	**G/G**	**0.02**	**9**	**G/A**	**0.17**	**42**	**A/A**	**0.81**	**0.89**
10	**rs7671250 C/T**	149364780	**11**	**C**	**0.11**	**93**	**T**	**0.89**	**1**	**C/C**	**0.02**	**9**	**C/T**	**0.17**	**42**	**T/T**	**0.81**	**0.89**
11	rs61760029 C/T	149364607	103	C	0.99	1	T	0.01	51	C/C	0.98	1	C/T	0.02	0	T/T	0.00	1.00
12	rs61760027 G/A	149363959	100	G	0.96	4	A	0.04	49	G/G	0.94	2	G/A	0.04	1	A/A	0.02	0.12
13	rs61760025 A/T	149363827	103	C	0.99	1	T	0.01	51	A/A	0.98	1	A/T	0.02	0	T/T	0.00	1.00
14	rs60660883 G/A	149363106	103	G	0.99	1	A	0.01	51	G/G	0.98	1	G/A	0.02	0	A/A	0.00	1.00
15	**rs6814934 C/G**	149362869	**54**	**C**	**0.52**	**50**	**G**	**0.48**	**12**	**C/C**	**0.23**	**30**	**C/G**	**0.58**	**10**	**G/G**	**0.19**	**0.44**
16	**rs7658048 C/T**	149362744	**66**	**C**	**0.63**	**38**	**T**	**0.37**	**18**	**C/C**	**0.34**	**30**	**C/T**	**0.58**	**4**	**T/T**	**0.08**	**0.16**

Notes: Allele- and genotype frequencies are based on 50 anonymous DNA samples. All SNPs were in Hardy-Weinberg Equilibrium (HWE; $p > 0.10$). The eight SNPs included in the three most common 5' UTR haplotypes are indicated in bold text.

Supplementary Table 3 Predicted transcription factor binding at eight common *MR* 5' UTR SNP sites

SNP	Allele	TRANSFAC	JASPAR
rs9992256 C/T	T	*RAP1*; *IRF1*	*FOXI1*; *NFIC (1x)*
	C	*Sp1*; *ICSBP*	*NFIC (2x)*
rs62332389 C/T	C	*Egr-1*; *Sp1*; *ER*; *v-Myc*	MZF1_5-13; HIF1A::ARNT; NFKB1
	T	*Sp1 (2x)*	MZF1_5-13; HIF1A::ARNT; NFKB1
rs5520 G/C	G	***Sp1 (3x)***	***SP1 (3x)***; *TFAP2A*; *HIF1A::ARNT*; ZNF354C
	C	***Sp1 (7x)***; *Ap-2*; *CACCC-bi*	***SP1 (6x)***; *TFAP2A (2x)*; ZNF354C
rs3216799 -/CT	-	**…**	*BRCA1*; *IRF1*
	CT	***HNF-1***	***HNF1B***; *FOXC1*; *FOXD1*
rs2248038 G/A	A	…	*ELK1*; *ELK4*; *SPIB*; TFAP2A
	G	…	TFAP2A; *NR3C1*
rs7671250 C/T	T	*Erg-1*; Oct-1	SRF; HOXA5; *NFIL3*
	C	Oct-1	*NF-kappaB*; *REL (2x)*; SRF; *TFAP2A*; HOXA5; *GATA2*
rs6814934 C/G	G	Sp1	*RORA_2*; *MZF1_5-13*; *MZF1_1-4 (2x)*; ELK1
	C	Sp1	*MZF1_1-4*; ELK1
rs7658048 C/T	C	…	*NFIC*; *YY1*; ETS1
	T	…	*FOXC1*; *REL*; ETS1

Notes: *In silico* analysis of predicted TF binding was performed using the TRANSFAC and JASPAR databases. Allele-specific TF binding is indicated in italic text, consistently predicted allele-specific TF binding is indicated in italic and bold text. Note that according to the JASPAR database the SNP rs2248038 influences a GRE-consensus sequence as indicated by allele-specific binding of NR3C1 (italic and underlined).



Supplementary Figure 1 Overview of linkage disequilibrium (LD) along 4 kb of the human *MR* promoter region and including the -2G/C and I180V SNPs in exon 2. Linkage correlations (r^2) and relative positioning of the SNPs are indicated.

Supplementary Table 4 Subject characteristics and *MR* SNP and haplotype frequencies according to sex in 450 elderly (study 1)

Variable	Total n= 450	Women n= 215 (47.8%)	Men n= 235 (52.2%)	*p*-value
Demographic				
Age, mean yrs (SD)	73.7 (5.7)	74.2 (5.9)	73.2 (5.5)	0.06
Dispositional optimism, mean (SD)	13.40 (4.68)	13.46 (4.69)	13.34 (4.68)	0.78
MR variants				
-2G/C, GG/CG/CC, freq.	0.28 / 0.47 / 0.25	0.24 / 0.49 / 0.27	0.32 / 0.45 / 0.23	0.15
I180V, AA/GA/GG, freq.	0.76 / 0.23/ 0.01	0.74 / 0.25 / 0.01	0.77 / 0.22 / 0.01	0.49
MR hap 1 -2G/180A, freq.	0.52	0.49	0.54	0.19
MR hap 2 -2C/180A, freq.	0.36	0.38	0.34	
MR hap 3 -2C/180G, freq.	0.12	0.13	0.12	

Supplementary Table 5 Subject characteristics and *MR* SNP and haplotype frequencies according to sex in 150 students (study 2)

Variable	Total group n= 150	Women n= 106 (70.7%)	Men n= 44 (29.3%)	*p*-value
Demographic				
Age, mean yrs (SD) *	23.9 (5.0)	23.3 (4.6)	25.4 (5.7)	**0.02**
LEIDS-R, mean (SD)				
Hopelessness/Suicidality *	4.9 (4.2)	5.5 (4.3)	3.4 (3.5)	**< 0.01**
Acceptance/Coping #	1.9 (2.3)	1.8 (2.3)	1.9 (2.2)	0.91
Aggression *	6.2 (4.3)	6.6 (4.4)	5.3 (4.1)	**0.05**
Perfectionism/Control *	7.1 (4.1)	7.7 (3.9)	5.5 (3.9)	**0.001**
Risk Aversion	8.5 (4.3)	8.9 (4.2)	7.6 (4.4)	0.08
Rumination	10.6 (4.5)	11.1 (4.3)	9.4 (4.9)	**0.04**
Total LEIDS-R	39.2 (17.3)	41.7 (16.9)	33.1 (17.0)	**< 0.01**
Symptoms of anxiety, depression				
HADS-anxiety, mean (SD)	6.2 (3.7)	6.92 (3.7)	4.57 (3.0)	**< 0.001**
HADS-depression, mean (SD) *	2.9 (3.2)	3.22 (3.5)	2.07 (2.0)	**0.05**
Total HADS, mean (SD) *	9.1 (6.2)	10.14 (6.5)	6.64 (4.6)	**0.001**
Never depressed, %	59.3	54.7	70.5	0.18
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)	**< 0.001**
MR variants				
-2G/C, GG/CG/CC, freq.	0.27 / 0.45 / 0.27	0.29 / 0.47 / 0.24	0.23 / 0.51 / 0.36	0.27
I180V, AA/GA/GG, freq.	0.76 / 0.23 / 0.01	0.74 / 0.24 / 0.02	0.80 / 0.20 / 0.00	0.59
MR hap 1 -2G/180A, freq.	0.50	0.53	0.43	0.10
MR hap 2 -2C/180A, freq.	0.37	0.33	0.47	
MR hap 3 -2C/180G, freq.	0.13	0.14	0.10	

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.

Supplementary Table 6 Subject characteristics and *MR* SNP and haplotype frequencies according to sex in 3523 patients with major depressive disorder and healthy controls (study 3)

Variable	Total n= 3523	Women n= 2312 (65.6%)	Men n= 1211 (34.4%)	*p*-value
Age, mean yrs (SD) #	43.9 (13.4)	42.7 (13.2)	46.1 (13.4)	**< 0.001**
Major depressive disorder, %	49.1	52.0	43.6	**< 0.001**
MR variants				
-2G/C, GG/CG/CC, freq.	0.23 / 0.53 / 0.24	0.24 / 0.53 / 0.23	0.22 / 0.52 / 0.26	0.23
I180V, AA/GA/GG, freq.	0.77 / 0.21/ 0.02	0.78 / 0.20 / 0.02	0.74 / 0.24 / 0.02	**0.008**
MR hap 1 -2G/180A, freq.	0.50	0.50	0.48	0.09
MR hap 2 -2C/180A, freq.	0.38	0.38	0.38	
MR hap 3 -2C/180G, freq.	0.12	0.12	0.14	

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

Supplementary Table 7 Results of linear regression analysis associating three *MR* haplotypes with dispositional optimism in study 1, or LEIDS-R, HADS and neuroticism scores in study 2, standardized regression coefficients (β), *p*-values and R^2 *change*

			MR hap 2		*MR* Hap 3		
			β	***p***	***β***	***p***	***R^2 change***
STUDY 1							
Optimism	**Total**	unadjusted	0.13	**0.007**	-0.01	0.88	0.02
		adjusted	0.12	**0.01**	-0.02	0.60	0.01
	Women	unadjusted	0.26	**< 0.001**	-0.00	0.95	0.07
		adjusted	0.24	**< 0.001**	-0.04	0.58	0.07
	Men	unadjusted	0.02	0.75	-0.01	0.85	0.00
		adjusted	0.01	0.89	-0.01	0.88	0.00
STUDY 2							
Hopelessness/Suicidality *	**Total**	unadjusted	-0.18	**0.03**	-0.05	0.55	0.03
		adjusted	-0.14	0.11	-0.07	0.42	0.02
	Women	unadjusted	-0.21	**0.04**	-0.08	0.45	0.04
		adjusted	-0.21	**0.04**	-0.07	0.52	0.04
	Men	unadjusted	0.01	0.97	-0.04	0.78	0.00
		adjusted	0.05	0.76	-0.09	0.56	0.01
Acceptance/Coping *	**Total**	unadjusted	-0.01	0.92	0.12	0.16	0.02
		adjusted	0.00	0.98	0.10	0.25	0.01
	Women	unadjusted	0.03	0.78	0.12	0.23	0.01
		adjusted	0.04	0.71	0.10	0.33	0.01
	Men	unadjusted	-0.10	0.52	0.13	0.40	0.03
		adjusted	-0.09	0.59	0.12	0.47	0.02
Aggression*	**Total**	unadjusted	-0.20	**0.02**	-0.01	0.89	0.04
		adjusted	-0.18	**0.04**	-0.01	0.88	0.03
	Women	unadjusted	-0.27	**0.008**	-0.05	0.62	0.07
		adjusted	-0.28	**0.007**	-0.03	0.77	0.07
	Men	unadjusted	0.01	0.93	0.03	0.87	0.00
		adjusted	0.04	0.83	0.00	1.00	0.00
Perfectionism/Control *	**Total**	unadjusted	-0.12	0.15	-0.08	0.34	0.02
		adjusted	-0.08	0.36	-0.09	0.30	0.01
	Women	unadjusted	-0.08	0.45	-0.06	0.59	0.01
		adjusted	-0.08	0.42	-0.04	0.70	0.01
	Men	unadjusted	-0.07	0.64	-0.18	0.25	0.04
		adjusted	-0.05	0.74	-0.21	0.20	0.04
Risk aversion	**Total**	unadjusted	-0.19	**0.03**	-0.14	0.10	0.04
		adjusted	-0.17	**0.05**	-0.15	0.09	0.04
	Women	unadjusted	-0.20	**0.05**	-0.12	0.22	0.04
		adjusted	-0.20	**0.05**	-0.11	0.28	0.04
	Men	unadjusted	-0.08	0.60	-0.22	0.16	0.05
		adjusted	-0.06	0.72	-0.25	0.13	0.06

Rumination	**Total**	unadjusted	-0.28	**0.001**	-0.10	0.20	0.08
		adjusted	-0.28	**0.001**	-0.08	0.34	0.07
	Women	unadjusted	-0.32	**0.001**	-0.14	0.15	0.10
		adjusted	-0.35	**< 0.001**	-0.09	0.38	0.11
	Men	unadjusted	-0.13	0.42	-0.06	0.70	0.02
		adjusted	-0.12	0.45	-0.07	0.68	0.02
Total LEIDS-R	**Total**	unadjusted	-0.25	**0.004**	-0.08	0.31	0.06
		adjusted	-0.22	**0.01**	-0.08	0.32	0.04
	Women	unadjusted	-0.26	**0.01**	-0.09	0.35	0.06
		adjusted	-0.27	**0.007**	-0.07	0.50	0.07
	Men	unadjusted	-0.10	0.51	-0.11	0.48	0.02
		adjusted	-0.08	0.61	-0.14	0.39	0.02
HADS anxiety	**Total**	unadjusted	-0.18	**0.03**	-0.10	0.25	0.03
		adjusted	-0.15	0.07	-0.08	0.32	0.02
	Women	unadjusted	-0.09	0.37	-0.09	0.36	0.01
		adjusted	-0.11	0.27	-0.05	0.65	0.01
	Men	unadjusted	-0.29	0.06	-0.13	0.39	0.09
		adjusted	-0.28	0.08	-0.15	0.34	0.09
HADS depression *	**Total**	unadjusted	-0.12	0.15	-0.09	0.31	0.02
		adjusted	-0.11	0.19	-0.06	0.47	0.01
	Women	unadjusted	-0.15	0.13	-0.14	0.16	0.03
		adjusted	-0.18	0.07	-0.09	0.38	0.03
	Men	unadjusted	0.03	0.83	0.05	0.76	0.00
		adjusted	0.06	0.73	0.02	0.88	0.00
Total HADS *	**Total**	unadjusted	-0.20	**0.02**	-0.09	0.26	0.04
		adjusted	-0.17	**0.04**	-0.08	0.36	0.03
	Women	unadjusted	-0.15	0.13	-0.12	0.24	0.03
		adjusted	-0.18	0.07	-0.06	0.54	0.03
	Men	unadjusted	-0.19	0.22	-0.05	0.76	0.04
		adjusted	-0.16	0.30	-0.08	0.61	0.03
Neuroticism	**Total**	unadjusted	-0.22	**0.008**	-0.07	0.40	0.05
		adjusted	-0.16	**0.05**	-0.08	0.30	0.02
	Women	unadjusted	-0.20	**0.05**	-0.10	0.32	0.04
		adjusted	-0.21	**0.04**	-0.08	0.47	0.04
	Men	unadjusted	-0.10	0.50	-0.07	0.64	0.02
		adjusted	-0.06	0.69	-0.12	0.44	0.02

Notes: Effect per haplotype allele was calculated as compared to the reference group (haplotype 1 carriers). Adjusted results are corrected for sex (in the total group) and age. Significant *p*-values are indicated in bold. * Transformed data.

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

Supplementary Table 8 Results of logistic regression analysis associating three 5' *MR* haplotypes with self-reported depression diagnosis in study 2 (**a**) and MDD diagnosis in study 3 (**b**), regression coefficients (*B*), standard errors (*SE*), *p*-values, odds ratios and 95% confidence intervals

a

		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	-0.53	0.35	.13	0.59 (0.30-1.16)	-0.32	0.41	.43	0.73 (0.33-1.61)
n= 150	adjusted	-0.65	0.37	.08	0.52 (0.25-1.08)	-0.06	0.43	.88	0.94 (0.40-2.18)
Women	unadjusted	-0.69	0.42	.10	0.50 (0.22-1.14)	-0.83	0.49	.09	0.50 (0.22-1.14)
n= 106	adjusted	-0.93	0.45	**.04**	0.40 (0.16-0.95)	-0.49	0.51	.34	0.61 (0.22-1.67)
Men	unadjusted	-0.15	0.73	.83	0.86 (0.20-3.61)	0.85	0.78	.27	2.35 (0.51-10.79)
n= 44	adjusted	-0.30	0.76	.69	0.74 (0.17-3.28)	1.12	0.83	.18	3.06 (0.60-15.51)

b

		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). (**a**) Data are presented for the total group as well as for women and men separately. Adjusted results are corrected for sex (in the total group) and age. Haplotype 2 associated with self-reported depression diagnosis among women according to a dominant model. (**b**) Data are presented for the total group, for women and men separately and for women of different age groups. MDD cases were mainly from the Netherlands Study of Depression and Anxiety (NESDA) cohort, healthy controls were mainly from the Netherlands Twin Registry (NTR). 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.