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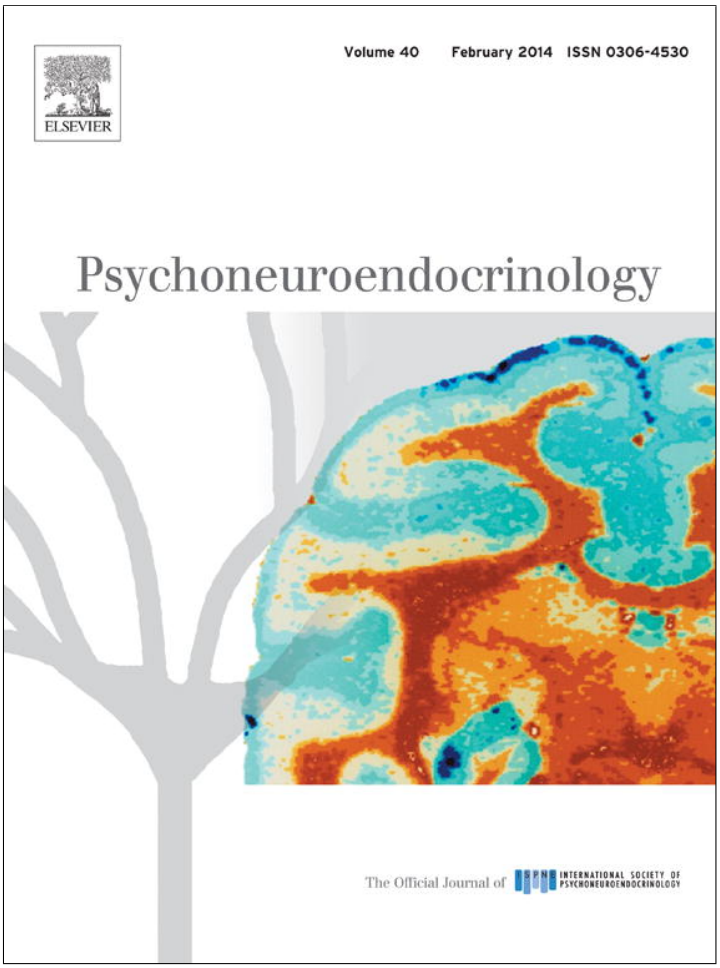

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Linking genetic variants of the mineralocorticoid receptor and negative memory bias: Interaction with prior life adversity

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Summary Substantial research has been conducted investigating the association between life adversity and genetic vulnerability for depression, but clear mechanistic links are rarely identified and investigation often focused on single genetic variants. Complex phenotypes like depression, however, are likely determined by multiple variants in interaction with environmental factors. As variations in the mineralocorticoid receptor gene (*NR3C2*) have been related to a higher risk for depression, we investigated whether *NR3C2* variance is related to negative memory bias, an established endophenotype for depression, in healthy participants. Furthermore, we explored the influence of life adversity on this association.

We used a set-based analysis to simultaneously test all measured variation in *NR3C2* for an association with negative memory bias in 483 participants and an interaction with life adversity. To further specify this interaction, we split the sample into low and high live adversity groups and repeated the analyses in both groups separately.

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NR3C2 variance was associated with negative memory bias, especially in the high life adversity group. Additionally, we identified a functional polymorphism (rs5534) related to negative memory bias and demonstrating a gene × life adversity interaction.

Variations in *NR3C2* are associated with negative memory bias and this relationship appears to be influenced by life adversity. As negative memory bias is implicated in the susceptibility to depression, our findings provide mechanistic support for the notion that variations in *NR3C2* — which could compromise the proper function of this receptor — are a risk factor for the development of mood disorders.
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1. Introduction

Mood disorders such as major depressive disorder (MDD) result in increased mortality risk and an immense burden for patients, their families, and society. The lifetime prevalence for depression in the US amounts to 16.5% (Kessler et al., 2005) and further increase is predicted (Mathers and Loncar, 2006). Thus, the search for risk factors for mood disorders is of particular importance (Collins et al., 2011).

To find genetic risk factors for heterogeneous and complex diseases such as MDD, the endophenotype approach has gained increasing recognition (Franke et al., 2009; Hasler et al., 2004). Endophenotypes represent heritable phenotypic constructs which are presumably more directly affected by genetic variations than disease categories or symptoms (Gottesman and Gould, 2003). In contrast to overt clinical phenotypes, they appear less complex and more homogenous (Kendler and Neale, 2010). Endophenotypes can be conceptualized and measured on different levels. For example, they can be found at the level of cell functioning, variations of brain function or structure, or at the level of behavior (Franke et al., 2009). Several endophenotypes have been proposed for MDD, among them negative memory bias (Beck, 2008; Hasler et al., 2004; Mathews and MacLeod, 2005), i.e. the tendency of depressed individuals to show enhanced memory for sad and pessimistic information. This bias forms a main cognitive risk and maintenance factor for MDD (Mathews and MacLeod, 2005), persists after remission (Leppanen, 2006) and is heightened in individuals with vulnerability to develop MDD (Chan et al., 2007; van Oostrom et al., 2013). Negative memory bias has also been associated with comparable structural brain variations as frequently found in MDD, i.e. increased amygdala volume and decreased hippocampal volume (Gerritsen et al., 2011).

Using negative memory bias as endophenotype for MDD, we investigated specifically whether genetic variation in a receptor for the stress hormone cortisol, the mineralocorticoid receptor (MR), is associated with higher vulnerability for MDD. MRs act together with glucocorticoid receptors (GRs) regulating the hypothalamus—pituitary—adrenal (HPA) axis, one of the major stress systems, which is altered in MDD (Joels et al., 2008). In the nuclear version, MRs have such a high affinity for glucocorticoids that they appear to be substantially activated even at baseline levels of cortisol (Joels et al., 2008). For a long time, research therefore primarily focused on the lower-affinity GRs (Anacker et al., 2011). However, it was recently shown that MRs also locate in the membrane of neurons. There, MRs appear to have a lower affinity, so that they respond to stress-induced increases of cortisol and play a functional role in mediating stress effects (Joels et al., 2008). In terms of psychopathology, brain MR expression is reduced in depressed patients (Klok et al., 2011a) and administration of MR agonists accelerates pharmacotherapeutic effects in MDD (Otte et al., 2010). Variations of the MR gene (also called nuclear receptor subfamily 3, group C, member 2, *NR3C2*) are associated with loss-in-function or reduced expression and have been related to hopelessness and higher MDD rates in pre-menopausal women (Klok et al., 2011b), neuroticism (DeRijk et al., 2011), HPA axis responsiveness (van Leeuwen et al., 2011) and higher amygdala reactivity (Bogdan et al., 2012).

Beyond genome-wide approaches, most previous studies investigating the influence of a candidate gene on MDD used single-SNP-based testing. More complex phenotypes like MDD or memory bias, however, are likely determined by several SNPs which each contribute small effects (Franke et al., 2009). Single-SNP analyses could therefore not optimally explain the heritability of such traits (Schwender et al., 2011). A newer approach is testing the combined effect of all genetic variations within a set of SNPs in a single analysis (Bralten et al., 2011; Deelen et al., 2011). This approach needs less power than genome-wide testing and also allows unbiased identification of SNPs within this set that were not yet known to be associated with the phenotype of interest.

It has repeatedly been shown for psychiatric disorders including MDD that genes might not link directly to disease, but instead genes modulate the vulnerability for such diseases. Thus, gene × environment interaction seems to be the prototypical mechanism leading to the development of several mental disorders (Caspi et al., 2003; Karg and Sen, 2012). This idea is in contrast to a genetic main-effect hypothesis that assumes the direct causation of a disorder by a specific genetic variation. The gene × environment interaction framework postulates that environmental factors cause disorders to occur and that genetic variants influence vulnerability as well as resilience to these factors, leading to psychopathology in some individuals only.

It is well established that the experience of life adversity is an important environmental factor in the etiology of MDD (Klengel and Binder, 2013). For example, a study by Kendler and colleagues demonstrated a causal relationship between stressful life events and the onset of MDD (Kendler et al., 1999). Cortisol has been implicated in mediating this interaction between life adversity and MDD (Wilkinson and Goodyer, 2011). The MR may play a particular role in this interaction as it

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was implicated in the stress response and feedback processes of the HPA axis (DeRijk and de Kloet, 2008; DeRijk et al., 2011), in emotional memory and anxiety (Brinks et al., 2009; Zhou et al., 2010). Thus, we set out to test whether variation in the MR as a receptor for the stress hormone cortisol could serve as a risk factor for the development of mood disorders, by probing the endophenotype negative memory bias. The association between negative memory bias and all SNPs genotyped in *NR3C2* was tested simultaneously in a large sample of healthy adults. We hypothesized a gene × life adversity interaction such that a potential association between *NR3C2* and negative memory bias would be stronger for individuals who had more life adversity.

2. Methods

2.1. Participants

The present behavioral study was part of the Brain Imaging Genetics (BIG) project conducted at the Donders Institute. Memory bias was assessed in 483 participants of BIG (62.3% females), aged 18–35 years (mean 22.4 years [SD = 3.16], Table 1) who volunteered to complete a web-based test battery. All participants were of European descent (Stein et al., 2012; Supplementary Table 7) and fluent in Dutch. They were screened using a self-report questionnaire for the following exclusion criteria: history of somatic disease potentially affecting the brain; current or past psychiatric or neurological disorder; medication (except hormonal contraceptives) or illicit drug use during the past six months; history of substance abuse; current or past alcohol dependence; pregnancy; lactation and menopause. Specific diagnostic interviews (including criteria for other disorders) were not part of BIG; however, as there is no evidence for clinically relevant psychiatric disorders in our participants, we refer to them as "healthy". BIG was approved by the regional medical ethics committee. All participants gave written informed consent and were financially compensated for participation.

2.2. Genotyping

Genetic analyses were performed as described in earlier publications from BIG (Bralten et al., 2011). DNA was extracted from saliva using Oragene kits (DNA Genotek, Kanata, Canada). Genotypes for *NR3C2* were available from genotyping using AffymetrixGeneChip SNP 6.0 (Affymetrix Inc., Santa Clara, CA) arrays. Quality control steps were equal to earlier publications on BIG (Bralten et al., 2011; Stein et al., 2012) except for the minor allele frequency, which was lower for those studies (0.01). We used a frequency cutoff of 0.05 as we are using a smaller subset of BIG. The call rate threshold for the arrays was set at 90% following recommendations of the manufacturer. SNPs were excluded if the call rate was <95% or they failed the Hardy–Weinberg equilibrium test ($p < 10^{-6}$ genome-wide). 346 SNPs within *NR3C2* and an additional 10-kb window on both sides (capturing regulatory sequences) were genotyped, of which 309 survived the quality checks. Participants were excluded if the call rate per individual was <95%.

Table 1 General characteristics of the study sample for both life adversity groups and sexes, including age, memory bias and life events.

	Low life adversity						High life adversity					
	Male (*N* = 108)			Female (*N* = 154)			Male (*N* = 74)			Female (*N* = 147)		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Age	22.0 (2.7)	18	33	21.7 (2.6)	18	34	22.8 (3.5)	18	35	23.1 (3.6)	18	33
Negative memory bias	.05 (.09)	.00	.40	.04 (.07)	.00	.44	.04 (.07)	.00	.40	.06 (.10)	0	.45
Positive memory bias	.34 (.25)	.00	1.00	.40 (.27)	.00	1.00	.32 (.22)	.00	.90	.39 (.25)	.00	.89
Number of life events	2.7 (1.1)	0	4	2.8 (0.9)	0	4	7.9 (3.0)	5	19	7.3 (2.8)	5	23

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2.3. Self-referent encoding/evaluation task (SRET)

Memory bias was assessed using a web-based version of the SRET (Hammen and Zupan, 1984), programmed in Flash (details see Gerritsen et al., 2011). During encoding, 12 negative and 12 positive trait adjectives (e.g. the Dutch words for "optimistic" or "unhappy") were presented one by one on a screen, in the same order for each participant. Participants were instructed to remember these words for a subsequent memory test, and asked to indicate whether each word was self-referent or not by pressing one of two buttons to ensure self-referent encoding. Following a distraction task of 2.5 min (mental arithmetic), participants had 3 min to freely recall and type in the studied adjectives they could remember. The two first and last adjectives on the encoding list were filler items and excluded from analyses to avoid primacy and recency effects, leaving 20 items in the analyses. Spelling errors were permitted since all responses that were not absolutely correct were checked manually. Three outcome variables were calculated: amount of adjectives recalled (overall memory performance), proportion of self-referent negative recall (negative memory bias) and proportion of self-referent positive recall (positive memory bias). The latter two variables were calculated by dividing the number of adjectives endorsed as self-referent and recalled in a given valence category by the total number of self-endorsed adjectives. These variables have the advantage of controlling for differences in overall rates of endorsement (Symons and Johnson, 1997). As especially negative memory bias had a skewed distribution, both memory bias measures were log transformed which resulted in reduced kurtosis and skewness (negative memory bias: skewness before and after transformation: 2.09 vs. 1.89, kurtosis: 4.34 vs. 3.14; positive memory bias: 0.05 vs. −0.25 and −0.83 and −0.98, respectively).

2.4. Life adversity

Life adversity was assessed retrospectively using an adapted version of the List of Threatening Life events developed by Brugha et al. (1985) in the same web-based test battery as the SRET (Gerritsen et al., 2011; van Oostrom et al., 2012). This inventory entailed life events which are likely to occur frequently and pose significant long-term threat. Participants had to indicate whether they had experienced a set of 21 specified life events (e.g. parental loss or sexual abuse) before age 16, after age 16 and within the last year. Life adversity was calculated by summing all experienced events over all categories. As this variable was not normally distributed, we log transformed the scores. For further analyses we stratified our sample according to the number of adverse events using a median split into high life adversity (above the median of four events, $n = 221$, 66.5% females) and low life adversity (maximally four events, $n = 262$, 58.8% females).

2.5. Gene-wide analysis

Association of variation in *NR3C2* with overall memory performance and memory bias was assessed with the set-based test with an additive genetic model in PLINK software, version 1.07 (Purcell et al., 2007). PLINK calculates a mean SNP statistic for each SNP set from the single SNP statistics of a specified maximum amount of independent SNPs (50) with a p-value smaller than 0.05 (Deelen et al., 2011). If the linkage disequilibrium (LD, calculated as r^2) between SNPs was higher than 0.8 (i.e. they were not independent), the SNP with the lowest p-value in the single SNP statistics was chosen per high-LD block. The analysis was then repeated with 10,000 simulated SNP sets for which the phenotype values were permuted over individuals using the mperm command in PLINK. Age and sex were included as covariates.

To test for a gene × life adversity interaction, we first pruned our data-set using the − indep command in PLINK as suggested in the manual. SNPs within a 50 SNP window that had $r^2 > 0.5$ (corresponding to a variance inflation factor [VIF] of two) with all other SNPs in the window were removed. This step removed 276 SNPs, leaving 33 in the analysis. Thereafter, we tested for a gene × life adversity interaction using the set-based analysis and adding life adversity (group membership) and the interaction term to the model. As it is not possible to obtain a whole-set p-value for this interaction, we calculated the likelihood of at least the amount of nominally significant SNPs given the null hypothesis, i.e. the probability to be significant per SNP being 0.05, using a binominal test. We repeated the interaction test using the continuous variable 'log transformed sum of life events' instead of 'group membership', since the median split on life events could create arbitrary differences between scores. To further specify the interaction results, we repeated the original set-based testing in the low and high life adversity groups separately.

2.6. Post hoc SNP based analysis

Following the gene-wide statistical approach, we examined the most promising SNP that showed significant associations with negative memory bias, using a general linear model (GLM) with age and sex as covariates. Again, additive genetic models were used. We tested for an interaction with life adversity using life adversity group membership as additional independent variable and repeated the analysis using the 'log transformed sum of life events' instead.

2.7. Haplotype analysis

Several haplotypes in *NR3C2* are known to be functional (e.g. DeRijk et al., 2011; Klok et al., 2011b; van Leeuwen et al., 2011). Thus, we also investigated whether common haplotypes are associated with negative memory bias: haplotype block 1: rs5522/rs2070951 in exon 2 and extending into the promoter region (Klok et al., 2011b) and haplotype block 2: rs5534/rs2871 in exon 9 of *NR3C2* (DeRijk et al., 2011). Individual haplotypes were reconstructed using the haplo.stats package (version 1.4.4) within R (version 2.12.0). Haplotypes were successfully reconstructed for all participants (posterior probabilities > 0.9). Separate GLMs were conducted to test the effects of having zero, one or two copies of each haplotype on negative memory bias.

Statistical testing was done using IBM® SPSS® Statistics 19.0.0. The significance level was set at $p = 0.05$ for all analyses and all tests were two-sided.

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3. Results

3.1. Gene-wide analysis

Table 1 displays general characteristics of our sample. *T*-tests and Mann–Whitney *U*-tests were conducted where appropriate to examine sex differences in age, positive memory bias, negative memory bias and number of life events. No differences between sexes emerged except for female participants having a stronger positive memory bias than males ($U = 22{,}979$, $p = 0.003$). As expected in a healthy population, participants endorsed on average 7 positive items and 1 negative item (ranges 2–10 and 0–10, respectively). They recalled on average 41% [SD 26.9] of the endorsed positive items, and 23% [SD 40.3] of the endorsed negative items. Across both valence groups, participants recalled significantly more items which they rated as self-descriptive: they successfully retrieved 42% of the items which were endorsed and 35% of the items which were not endorsed ($p < 0.001$).

Analyzing the gene-wide association using set-based testing showed an association for negative memory bias ($p = 0.017$) with 27 SNPs having *p*-values < 0.05 of which six were independent (see Table S1 for more information). There was no association with positive memory bias or overall memory performance (both $p > 0.5$). To address the potential concern that our sample size might have been too small to detect linear effects using a minor allele frequency of 0.05, we repeated the analysis with frequency cutoffs of 0.10 and 0.15, which did not change the results.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.11.010.

Testing for an interaction with life adversity (group membership) resulted in six out of 33 SNPs showing a significant interaction (see Table S2). A binominal test revealed that the likelihood for at least six SNPs being significant given the null hypothesis, is $p = 0.005$. We repeated the analysis using the log transformed sum of life events as dependent variable revealing a similar result (five SNPs significant, $p = 0.023$). Analyzing the high and low life adversity group separately resulted in a significant association in the high life adversity group ($p = 0.0004$, 47 SNPs significant, 14 independent, Table S1) but not in the low life adversity group ($p > 0.8$, four SNPs significant, three independent).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.11.010.

3.2. Post hoc SNP-based analysis

Fig. 1 displays SNP-by-SNP associations between *NR3C2* and negative memory bias. Two significant SNPs, rs5534 and rs2871, were located in coding regions. As rs5534 (frequency of the minor allele A: 0.442) has been shown to be functional (Nossent et al., 2011), we focused on this SNP as a promising marker for negative memory bias. In our sample, 166 participants were homozygous for the G allele, 226 participants were heterozygous, and 91 participants were homozygous for the A allele (Table 2). rs5534 was associated with negative memory bias ($F = 4.526$, df = 2, $p = 0.011$) and post hoc tests showed that participants with the AA genotype had an almost 100% stronger negative memory bias than both AG and GG ($p = 0.018$ and $p = 0.003$, respectively; Fig. 2) with no difference between the latter two groups ($p > 0.3$). This SNP was not associated with positive memory bias or memory performance (both $p > 0.5$).

We found a significant rs5534 × life adversity (group membership) interaction on negative memory bias ($F = 9.838$, df = 2, $p < 0.0001$). Using the log transformed sum of life events as the independent variable did not change the result ($F = 2.26$, df = 26, $p = 0.0005$). Post hoc tests indicated that the genetic groups did not differ in negative memory bias if participants had low life adversity (all $p > 0.15$, Fig. 3). However, within the high life adversity group, participants homozygous for the A allele of rs5534 had a stronger negative memory bias than both other groups (both $p < 0.0001$), with no difference between the latter two groups ($p > 0.5$). Furthermore, participants with the AA genotype of rs5534

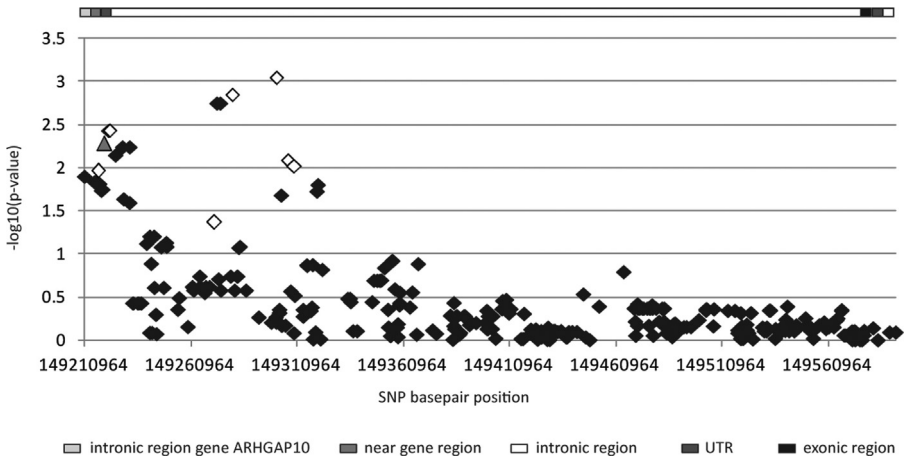

Figure 1 Gene-wide and SNP-by-SNP associations between *NR3C2* gene variants and negative memory bias. *Note*: gene-wide significance was observed using PLINK. SNP: single nucleotide polymorphism, UTR: untranslated region. SNPs which were used to calculate the gene-wide *p*-value are indicated by open diamonds, rs5534 is shown as gray triangle, other SNPs are depicted as black diamonds. SNP function is indicated by gray shaded bars according to http://www.scandb.org/.

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Table 2 General characteristics of the rs5534 genotype groups.

Genotype	GG (N = 166)			AG (N = 226)			AA (N = 91)		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Age	22.8 (3.4)	18	35	22.0 (2.8)	18	33	22.5 (3.5)	18	34
Negative memory bias	.04 (.07)	0	.44	.04 (.08)	.0	.45	.07 (0.11)	.0	.40
Positive memory bias	.38 (.26)	0	1	.38 (.27)	0	1	.34 (.24)	0	1
Number of live events	4.7 (2.7)	0	17	5.0 (3.3)	0	23	5.3 (3.4)	1	16

and high life adversity had a stronger negative memory bias than participants of the same genotype but low life adversity (p = 0.0002). Within the other genetic groups, negative memory bias did not differ between life adversity groups (both $p > 0.7$).

3.3. Haplotype analysis

Three haplotypes were observed in block 1 (rs2070951/rs5522), with frequencies as reported previously (DeRijk et al., 2011): haplotype1 (G-A) was most common (52%), followed by haplotype2 (C-A, 37%) and haplotype3 (C-G, 11%). No haplotype was related to negative memory bias (all $p > 0.6$).

Four haplotypes were found in block 2 (rs5534/2871), with similar frequencies as reported previously (DeRijk et al., 2011): haplotype1 (A-G) was most present (57%), followed by haplotype2 (G-A, 31%), haplotype3 (G-G, 11%) and haplotype4 (0.4%). As haplotype4 had such a low frequency, we limited our analyses to haplotype1, 2 and 3.

Haplotype1 was significantly associated with negative memory bias (F = 4.218, df = 2, p = 0.015). Post hoc comparisons showed that participants with no copy of haplotype1 had a stronger negative memory bias than participants with one or two copies (p = 0.029 and p = 0.004, respectively). Furthermore, there was a trend for an association between haplotype2 and negative memory bias (F = 2.961, df = 2, p = 0.053). Post hoc comparisons showed that participants with no copy of haplotype2 had a smaller negative memory bias than participants with two copies of haplotype2 (p = 0.029).

Finally, both haplotype1 and haplotype2 showed an interaction with life adversity (group membership; F = 10.435, df = 2, $p < 0.0001$ and F = 6.828, df = 2, p = 0.001, respectively). Post hoc tests revealed that in the low life adversity group, participants with different haplotype1 or haplotype2 frequencies did not differ in negative memory bias (all $p > 0.1$). However, in the high life adversity group, participants with no copy of haplotype1 had a stronger negative memory bias than participants with one or two copies of this haplotype (both $p < 0.0001$). Additionally participants with two copies of haplotype2 in the high life adversity group had a stronger negative memory bias than participants with either one or no copy of haplotype2 (p = 0.003 and p = 0.001, respectively).

No other comparison in block 2 was significant (all $p > 0.1$).

4. Discussion

This study provides initial evidence that variation in *NR3C2* is related to negative memory bias in healthy adults, especially given a history of life adversity. As negative memory bias is an endophenotype for MDD, our finding supports the notion that common *NR3C2* variation is a risk factor for developing mood disorders (DeRijk and de Kloet, 2008), especially in combination with life adversity.

Additionally, we found a functional variation as a potential marker for heightened MDD risk, rs5534, which was associated to negative memory bias and showed an interaction with life adversity. This coding SNP in exon 9 of *NR3C2*

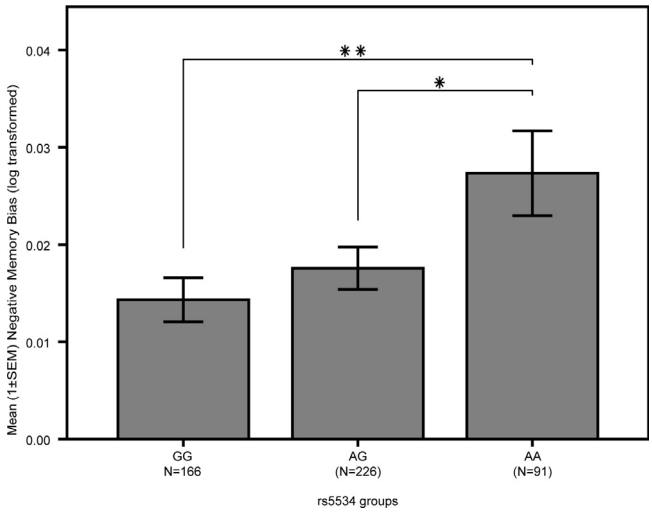

Figure 2 Comparison of negative memory bias between participants with genetic differences in rs5534. *Note*: *$p < 0.05$, **$p < 0.01$.

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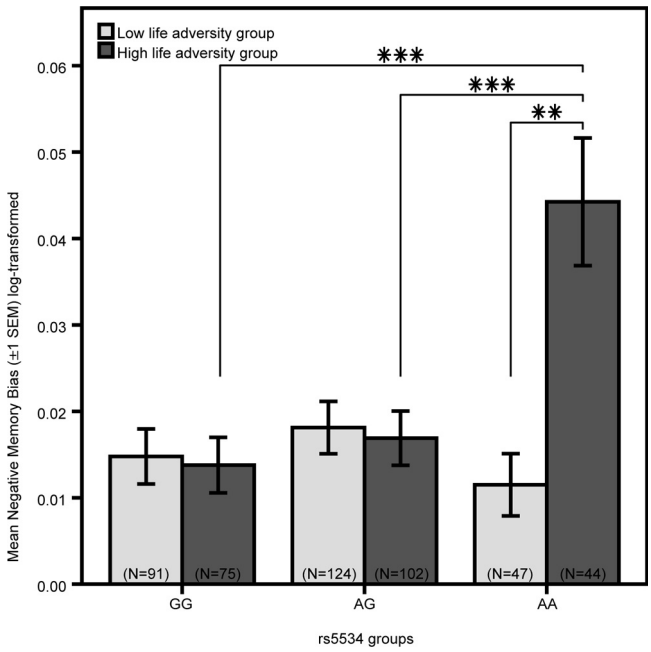

Figure 3 Interaction of rs5534 and life adversity on negative memory bias. Note: $^{**}p < 0.01$, $^{***}p < 0.001$.

(van Leeuwen, 2010) was associated to a reduced efficiency of an upstream binding site for microRNA hsa-miR-383 in two human cell lines (Nossent et al., 2011). In vitro, the A allele of rs5534 led to increased microRNA-induced repression of MR expression (Nossent et al., 2011). This SNP could thus be a marker for impaired regulation of MR expression which might in turn result in dysfunctional changes of cortisol regulation after stressful events. Furthermore, impaired regulation of MRs could influence excitability and structural integrity of amygdala and hippocampus (Gass et al., 2000; Groeneweg et al., 2011) whose pathophysiology has been associated with mood disorders (Bremner et al., 2000; Frodl et al., 2002) and negative memory bias (Gerritsen et al., 2011).

The significant post hoc main effect for rs5534 itself was expected given the significant finding in the gene-wide analysis, and thus its *p*-value is putatively inflated (Kriegeskorte et al., 2009). However, we want to emphasize that rs5534 was chosen as a promising marker, because of an earlier report of its functionality (Nossent et al., 2011). It forms a haplotype with the second exon SNP that was associated with negative memory bias (rs2871) and this haplotype was associated with negative memory bias. This association seems counterintuitive concerning an earlier finding where two copies of haplotype2 were associated with lower neuroticism scores in a sample of 100 patients with MDD or anxiety disorders (DeRijk et al., 2011). However, this same study found no association of haplotype2 and neuroticism in healthy controls. More research is certainly needed to understand the role of the functional haplotype blocks in *NR3C2* and their relation to psychopathology.

Earlier publications on variations in *NR3C2* have found two other SNPs, rs5522 and rs2070951, and their haplotypes to be associated with reward learning, HPA axis functioning, hopelessness, increased depressive symptoms in individuals above age 85 and pre-menopausal women (see e.g. DeRijk et al., 2011; Klok et al., 2011b; Kuningas et al., 2006). We did not find any associations of these SNPs or haplotypes in our study in line with several previous publications (Bouma et al., 2011; Supriyanto et al., 2011; Velders et al., 2011).

We found a gene × life adversity interaction such that associations between genetic variants and negative memory bias were highly pronounced in individuals with high life adversity, who, in general, are more likely to develop mood disorders (Kendler et al., 1999). So far, *NR3C2* has not directly been implicated in the risk for MDD in large scale GWAS studies (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013). This missing association may have many reasons, one of those might be the role life adversity seems to play in linking risk genes to the clinical outcomes. Our finding thus supports the importance of gene × environment interactions in the search for risk factors for psychiatric disorders such as MDD.

As an emotionally negative cognitive bias might also affect recall of negative events from autobiographical memory, causality is difficult to assign. However, the questionnaire used to assess life events limits the effects of recall biases, because it asks for the occurrence of several factual and specific events, like divorce or death of parents that require a clear yes or no answer (Brugha et al., 1985). Certainly, depressed or sad individuals recall more negative events from autobiographical memory, but these negative recalled events tend to be overgeneralized and not factual or detailed in time and place (King et al., 2010). It is therefore very unlikely that negative memory bias will affect recall of the kind of factual information asked for in our study. However, only prospective longitudinal studies will allow drawing definite conclusions regarding causality.

Several reports have also related variations of *NR3C2* to pseudohypoaldosteronism type 1 and hypertension (Geller et al., 1998; Riepe et al., 2006, 2004; van Leeuwen, 2010). Unfortunately, we do not have data on blood pressure or

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aldosterone levels available for our cohort and to the best of our knowledge, no study has investigated the relation between blood pressure or aldosterone levels on one hand with emotional memory bias on the other hand. More studies are certainly needed to reveal possible associations.

It could be criticized that life events and memory bias were assessed using a web-based test battery. This approach allowed the participants to determine themselves when and where to do the test and offers less organizational and time constraints, but it gave less control over the experimental setting, for example time of day. However, more and more studies are appearing using web-based tests and surveys. For example, a recent study using cognitive and perceptual tasks demonstrated that data acquired using web-based testing is comparable in reliability and validity to data from laboratory tests, even if the participants are unsupervised (Germine et al., 2012). Furthermore, we mitigated potential variance by the focus on relative measures (memory bias) instead of absolute measures (memory performance), because we can assume that recall of both negative and positive words is affected in the same way. Finally, it is unlikely that noise potentially introduced by the web-based approach is affecting a certain genotype more than another. Thus, the web-based approach allowed us to test a large sample of subjects for self-referenced negative memory bias with putatively sufficient reliability and validity.

It could also be argued that our sample was rather young and might thus have experienced few life events. However, a comparison with other studies using the original version of the List of Threatening Life Events (Brugha et al., 1985) contradicts this argument: a recent study in over 1000 Dutch participants (mean age 53.5 years) found not only satisfactory construct validity and stability over two years, but also a median lifetime event score of 5 and a maximum of 21, which is very similar to our study (Rosmalen et al., 2012).

A particular strength of our study was the gene-wide statistical approach, which takes into account all observed variation in *NR3C2* simultaneously. Especially for complex phenotypes that might depend on several genetic factors and where possible allelic heterogeneity makes it difficult to find associations, this approach can increase statistical power (Bralten et al., 2011; Deelen et al., 2011; Schwender et al., 2011). Another strong point of this study is the fact that it was done in a sample of healthy participants so that the associations are not influenced by psychopathology, chronic disease processes, or therapy. Rather, we report a relationship between genetic variation with an established cognitive endophenotype for MDD, which might predispose individuals to develop MDD later in life.

To the best of our knowledge, this is the first study that investigated the effect of common variants in *NR3C2* on negative memory bias using a gene-wide approach. Variation in *NR3C2* was associated with this cognitive endophenotype, especially in participants with high life adversity. Common variations in *NR3C2* — which may compromise proper functioning of the receptor — could thus be a risk factor for developing mood disorders, especially in combination with life adversity. Our results extend earlier findings that MRs might be critically involved in the pathophysiology of mood disorders, and may thereby provide new avenues for pharmacological interventions.

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Conflict of interest statement

All authors declare no conflict of interest.

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