Cortisol exposure, cognition and clinical course of bipolar disorder

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5. Glucocorticoid and mineralocorticoid receptor polymorphisms and clinical characteristics in bipolar disorder patients

Authors

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Abstract

Introduction

The Hypothalamus-Pituitary-Adrenal (HPA)-axis is often found to be dysregulated in Bipolar Disorder (BD) while stress and changes in day-night rhythms can trigger a new mood episode. Genetic variants of the Glucocorticoid Receptor (GR)- and Mineralocorticoid Receptor (MR)-gene influence both the reactivity of the stress-response and associate with changes in mood. In this study we tested the hypothesis that these polymorphisms associate with different clinical characteristics of BD.

Method. We studied 326 outpatients with BD and performed GR genotyping of the *TthIII*I, ER22/23EK, N363S, *Bcl*I, and 9β polymorphisms, as well as MR genotyping of the 2G/C and I180V variants. All patients were interviewed for clinical characteristics.

Results. Seasonal patterns of hypomania are related to the *Bcl*I haplotype and the TthIIII+9β haplotype of the GR gene (respectively crude p=.007 and crude p=.005). Carriers of the ER22/23EK polymorphism had an almost 8 years earlier onset of their first (hypo) manic episode than non-carriers (crude p=.004, after adjustment p=.016). No evidence for a role of the MR in modifying clinical manifestations was found.

Conclusion. Polymorphisms of the GR–gene are factors which influence some clinical manifestations of BD, with respect to seasonal pattern of (hypo) mania and age of onset.

1. Introduction

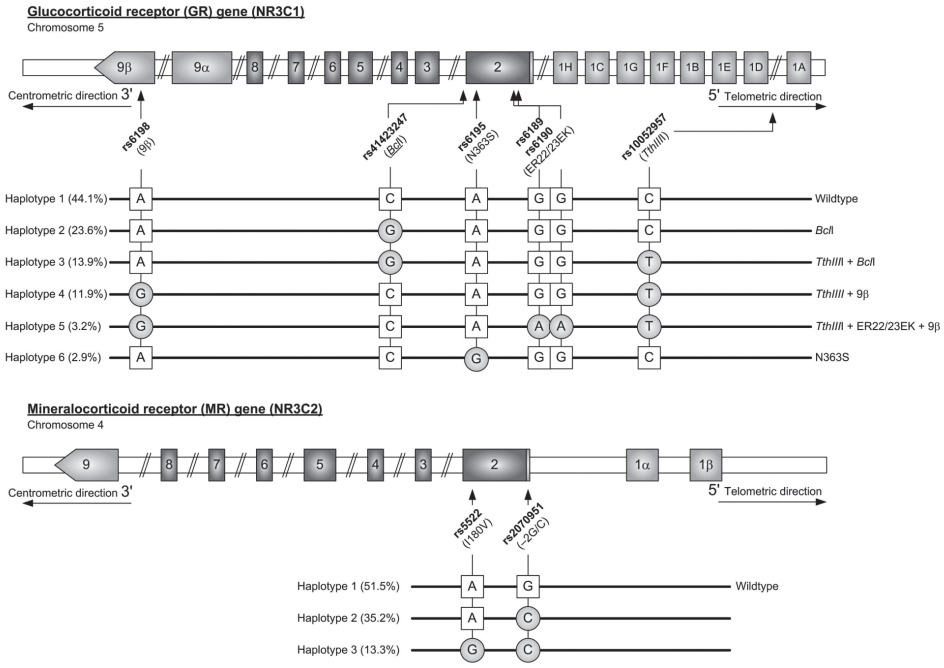
Bipolar disorder (BD) is a common mood disorder with a prevalence of around 2% in the general population. Several subtypes are distinguishable, of which Bipolar Disorder type 1 (BD1) is more prevalent than Bipolar Disorder type 2 (BD2*)* (1). BD1 is characterized by at least 1 manic episode and BD2 is characterized by at least one hypomanic episode and one depressive episode. The course and characteristics of the disease are highly variable and unpredictable. A clinically remarkable characteristic of BD are seasonal patterns of mood episodes in around 25% of all BD patients (2). Currently known predictors of an adverse course and higher severity include incomplete recovery between episodes, a history of rapid cycling and more than 10 episodes in the medical history, a history of childhood abuse, co-morbid panic disorder, substance abuse (3) and suicide attempts (4).

Stress and (abrupt) changes in day-night rhythms are common triggers of new mood episodes (5-7). The Hypothalamic Pituitary Adrenal (HPA)-axis is a central component of the stress-response and there is ample evidence that in BD the HPA-axis is dysregulated (8, 9). The most prominent finding is hypercortisolism in challenge tests like the Dexamethason Suppression Test (DST) and the Dexamethason/ Corticotropin Releasing Hormone (DEX/CRH)-Test. Moreover, hypercortisolism after the DEX/CRH-test is found during depressive episodes, manic episodes, as well as during remission, as compared to healthy controls (Schmider et al.,to healthy controls and family members (10), indicating that hypercortisolism is a feature predominantly observed during the stress responses and during active disease, but not in basal conditions.

The cortisol signal is mediated by two receptors, the low affinity Glucocorticoid Receptor (GR) and the high affinity Mineralocorticoid Receptor (MR). Under non-stress conditions, the low levels of cortisol already occupy a substantial number of the MR and GR, while only during stressful situations high levels of cortisol predominantly bind to the GR (11, 12). The activity of both these receptors regulates the stress-response, including the HPA axis and the autonomic output (13-15). Furthermore, the clinical relevance of these findings has become clear in the therapeutic effect of GR-antagonists, like mifepristone, improving mood and neurocognitive functioning in bipolar depression (16). Administration of the MR-agonist fludrocortisone as add-on to antidepressants greatly speeded up treatment response in depressed patients (17). The mood stabilizers lithium and valproate may mediate their therapeutic effects by up regulation of central GR (18) and by influencing GR-co-chaperone proteins (19).

Several polymorphisms of the GR- and MR-gene are involved in regulating the HPA-axis and may contribute to differences in the sensitivity of the stress-response (12, 20, 21), they are summarized in Figure 1.

Figure 1. *Schematic overview of the GR and MR gene structures with their respective most frequent haplotypes. The orientation of the gene is marked by arrows, with the genes transcribed from left to right. Some exons are not translated into protein (light gray). The gene encoding the GR consists of 17 exons, with the untranslated exons 1A-1H and 9a and 9b resulting in different mRNA splice variants. The five SNPs (rs 10052957 [Tth///I], rs6189/rs6190 [ER22/23EK], rs6195 [N363S], rs41423247 [BclI], and rs6198 [9β]) that were genotyped are indicated with arrows. Six main haplotypes are known (52). The gene encoding the MR consists of ten exons, exon 1α till exon 9. The two SNPs that were genotyped are indicated with arrows. The functional MR -2 G/C SNP (rs2070951) is located in exon 2, two nucleotides before the first translation start site. The functional MR I180V SNP (rs5522) is located in exon 2 and results in an Isoleucine to Valine amino-acid change. Both SNPs are located in a haplotype bin that extends into the promoter region. Three main haplotypes are known (53).*



GR polymorphisms have repeatedly been associated with mood disorders; the *Bcl*I and the ER22/23EK polymorphisms of the GR gene and, to a lesser extent the 9β polymorphism, may be most prominently associated with psychopathology (for review: (22)). In addition, two polymorphisms in the MR gene, the I180V SNP and the -2G/C SNP, have recently been found to be associated with the regulation of the stress-response in healthy subjects (13, 23). The I180V SNP was found to be associated with a higher frequency of depressive symptoms in an elderly cohort of participants aged 85 years and above (24) and with neuroticism in adult depressed patients (25).

Together these findings suggest a role of these GR- and MR-gene polymorphisms in the course and severity of bipolar mood disorders. To our knowledge no clinical study focused on the relationship between GR and MR polymorphisms and clinical characteristics of BD. We investigated whether different polymorphisms are associated with different clinical manifestations of BD.

2. Materials and Methods

2.1 Study design

This is a cross-sectional study in patients with BD. The study protocol was approved by the Medical Ethical Committee in Utrecht (METiGG) and has been carried out in accordance with the Declaration of Helsinki. All participants signed for informed consent.

2.2 Study population

All 702 patients, diagnosed and treated in the Department of Mood Disorders, PsyQ The Hague, with BD type 1, BD type 2, cyclothymia (a cyclic pattern of mood episodes which do not completely fulfill DSM-IV criteria), or bipolar disorder Not Otherwise Specified according DSM-IV-TR diagnosis, and older than 18 were selected to be asked to participate in this study. They were requested during a time frame of two years, by their psychiatrist and psychologist or by letter. Of these 702 patients 328 patients (46.7%) who responded and agreed to participate were enrolled in the study. The other 374 patients did not respond for largely unknown reasons, as they did not reply on repeated invitation by letter. Two more participants were excluded from the analysis, because only genotypic data and no clinical data were collected. Therefore, the present analyses were based on 326 (46.4%) subjects, yet some variables were missing in some participants due to logistical reasons. The included 326 patients did not differ from the 304 non-participating patients with respect to gender (39.9% male versus 41.4%

male respectively, p=.53). The included patients differed from the non-responders with respect to BD subtype (80% BD1, 19% BD2 and 1% BD NOS/cyclothymia versus 68% BD1, 26% BD2 and 6% BD NOS/cyclothymia, respectively, p<.001) and with respect to age (mean 48.0 ± 11.2 years versus 45.7 ± 12.5 respectively, p=.02) .

Data on seasonal patterns were complete in 301 (92.3%) subjects for depressive episodes, 298 (91.4%) subjects for manic episodes and 295 (90.5%) subjects for hypomanic episodes. Data on number of episodes and age of onset for mania were complete in 289 (88.7%) subjects and for depressive episodes in 292 (89.6%) subjects. Data on BD subtype were complete in 309 (94.8%) subjects. Age, gender and the presence of an anxiety disorder were assessed in all participants.

2.3 Assessments

All patients were diagnosed with bipolar disorder by a psychiatrist during their first visit on our Department. For the purpose of this study they were subsequently interviewed by one out of three trained psychologists who collected sociodemographic and disease characteristics, under supervision of a psychiatrist. Diagnostic status according to the DSM-IV-TR was assessed with a standardized diagnostic interview the Dutch version of the MINI International Neuropsychiatric Interview Plus (version 5.00-R; MINI-PLUS (26)). The age of onset of first symptoms of either (hypo)mania or depression, the number of episodes of (hypo)mania and depression, and the presence of co morbid anxiety disorders (according DSM-IV-TR) were assessed with the MINI. Psychiatric co-morbidity was assessed with the MINI and included lifetime and current anxiety disorders, somatoform disorders, eating disorders, and substance disorders. Current anxiety disorder was summarized into one dichotomous variable representing the presence or absence of any anxiety disorder. Suicide risk was classified according to the MINI in low, moderate and high risk.

Bipolar Disorder was further characterized by the Questionnaire for Bipolar Illness-Dutch Translation (QBP-NL) (27, 28). The QBP-NL BD was used to subtype BD into BD1, BD2, cyclothymia or BD Not Otherwise Specified (BD NOS). Due to small numbers the last two variables were aggregated in BD NOS. All patients were questioned in detail about seasonal patterns of depressive, hypomanic and manic episodes with the QBP-NL. Mood episodes were classified according to seasonal patterns, which was defined as whether the onset of the episode (i.e., depressive, hypomanic, or manic) was predominantly in the same season (winter, spring, summer or autumn). Age of onset and number of episodes were nominal variables, all other variables were categorical.

2.4 DNA analysis

From all 326 patients, blood samples of 40 ml were drawn with standard venapuncture techniques, collected in EDTA tubes, to analyze GR and MR gene polymorphisms (see Figure 1). Genomic DNA was isolated from fresh blood samples using the Puregene whole blood DNA-extraction kit (Gentra Systems Inc; MN). Genotyping was performed through PCR-based techniques. GR genotyping was performed in the Laboratory of Endocrinology of the Erasmus MC Rotterdam. MR genotyping was performed by Department of Pharmacology in Leiden in collaboration with the aforementioned laboratory in Rotterdam. Allelic discrimination was performed to genotype the subjects, using TaqMan Universal PCR master mix, primers and probes (Applied Biosystems, Nieuwerkerk aan den IJssel, Netherlands) and a Taqman ABI Prism 7900 HT Sequence Detection System as previously described (15, 29). Reaction components and amplification parameters were based on the manufacturer's instructions using an annealing temperature of 60°C and optimized concentrations for primers of 400 nmol/L for each polymorphism. The genotypes of all heterozygous and homozygous carriers of the polymorphisms were re-analyzed and yielded identical genotypes.

GR genotyping was completed for *TthIII*I in 307 (94.2%) subjects, for the ER22/23EK in 310 (95.1%) subjects, for N363S in 313 (96.0%) subjects, for *Bcl*1 in 310 (95.1%) subjects and for 9**β** in 314 (96.3%) subjects. MR genotyping was completed for -2G/C in 302 (92.6%) subjects and for I180V in 305 (93.6%) subjects. Due to small numbers, frequencies of the ER22/23EK (GA=17, AA=2), N363S (AG=20, GG=0) and I180V (AG=67, GG=10) Single Nucleotide Polymorphisms (SNPs) were combined into one group for the hetero- and homozygote carriers of the minor allele.

2.5 Statistical analysis

Haplotypes were created with SNPHAP (version 1.3, www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt). All SNPs were tested for Hardy-Weinberg equilibrium. Thesias (Tregouet & Garelle, 2007) was used to assess inter-marker linkage disequilibrium scores (LD scores), expressed as D'. All further analyses were performed with Statistical Packages for Social Sciences (SPSS) version 17.

For analyses of baseline data t-tests and chi-square tests were used, when appropriate. All variables were normally distributed, except for "number of manic episodes" and "number of depressive episodes". These two positively skewed variables were therefore log transformed in all analyses and back-transformed geometric means values were presented in our results. Analyses of disease characteristics in relation to SNPs and haplotypes (see Figure 1) were performed with t-tests, Analysis of Variance (ANOVA)

and Chi Square tests. Adjustment for age, sex and subtype of BD was performed with Analysis of Covariance (ANCOVA) and multivariable logistic regression analysis. Results were considered statistical significant with p-value < 0.01 for GR SNPs and haplotypes and with a p-value <.025 for MR SNPs and haplotypes, to take multiple testing into account for SNP analyses. Post hoc Dunnett's method was used in post hoc tests to correct for multiple testing in haplotype analyses.

3. Results

3.1 Patient characteristics

Sociodemographic and disease characteristics are summarized in Table 1. In total 326 patients were enrolled in this study, of whom 80% were diagnosed with BD1, 19% with BD2, and 1% with BD NOS. Age ranged from 18-80 years, with a mean of 48 years (SD 11.2). In a total of 94 patients (31.2%) a seasonal pattern was present. Of all patients with seasonal depressive episodes, 87% had autumn and/or winter episodes. Eighty per cent of all seasonal manic episodes and 93% of all seasonal hypomanic episodes were spring/summer episodes. In further analyses seasonal patterns were dichotomized for hypomanic, manic and depressive episodes.

3.2 Genotypes and haplotypes

All SNPs were in Hardy-Weinberg equilibrium, except for ER22/23EK (χ^2 = 8.15, p<.01). The frequencies of the GR and MR Single Nucleotide Polymorphisms (SNPs) are presented in Tables 2 and 3. Comparisons between the GR SNPs resulted in different LD scores, D' ranged from 0.2-1.0 with an average of 0.9. Linkage of the two MR SNPs was high, with D'=1.0. Haplotypes created with SNPHAP was successful in 99.9% for the GR gene and 100% for the MR gene. Haplotypes are described in Figure 1, and numbering as used in Table 4 is defined there. Both GR and MR haplotypes 1 were the most prevalent wild-type haplotypes, and were therefore used as the reference category in further analyses. Age and sex distributions did not differ among the GR and MR haplotypes.

***Table 1**. Demographic and clinical characteristics of 326 BD patients*

Variable	Total group
No. (n)	326
Male n(%)	130 (39.9)
Age (yr); mean (SD)	48.0 (11.2)
Bipolar disorder subtype; n (%)	
Type 1	246 (80)
Type 2	60 (19)
NOS	3 (1)
Rapid cycling; n(%)	84 (27.2)
Seasonal pattern; n(%)	94 (31.2)
Depressive episodes	83 (27.6)
Manic episodes	41 (13.9)
Hypomanic episodes	28 (9.6)
Current drug use; n(%)	28 (9)
Current alcohol use ≥3/day; n(%)	32 (16)
Psychiatric co morbidity; n(%)	
Current co morbid disorder	118 (36)
Current anxiety disorder	80 (25)
Suicide risk moderate/ high	43 (13)
Physical morbidity; n(%)	
Cardiovascular disease	26 (10)
Endocrine disease	58 (22)
Medication use; n(%)	
Lithium	259 (80%)
Valproate	36 (11%)
Antidepressant	91 (28%)
Number of depressive episodes; median (IQR)	5.5 (3.0-15.0)
Number of manic episodes; median (IQR)	5.0 (2.0-12.0)
Age of onset of depression; mean (SD)	25.6 (11.7)
Age of onset of mania; mean (SD)	29.3 (11.8)

IQR denotes interquartile range.

Table 2. Association of GR and MR gene polymorphisms with co-morbid anxiety disorder and seasonal pattern in 326 BD patients

	Geno-type	N(%)	Current anxiety disorder		Seasonal pattern:					
				p	Depres-sion	p	Mania	p	Hypoma-nia	p
GR SNP										
rs10052957 (Tth///I)	CC	155 (50.5)	36 (23.2)	.12	39 (26.7)	.64	18 (12.7)	.67	10 (7.1)	.25
	CT	118 (38.4)	25 (21.2)		34 (31.5)		15 (13.9)		14 (13.2)	
	TT	34 (11.1)	13 (38.2)		8 (25.0)		6 (18.8)		4 (12.5)	
rs6189 (ER22/23EK)	GG	291 (93.9)	73 (25.1)	.05	78 (28.7)	.32	36 (13.5)	.70	28 (10.6)	.15
	GA+AA	19(6.1)	1 (5.3)		3 (17.6)		3 (16.7)		0	
rs6195 (N363S)	AA	293 (93.6)	70 (23.9)	.91	80 (29.5)	.06	38 (14.3)	.59	27 (10.2)	.48
	AG	20(6.4)	5 (25.0)		2 (10.0)		2 (10.0)		1 (5.3)	
rs41423247 (Bc/1)	CC	125 (40.3)	27 (21.6)	.74	28 (24.1)	.29	17 (15.0)	.70	7 (6.2)	.05
	CG	138 (44.5)	35 (25.4)		38 (29.5)		16 (12.3)		13 (10.2)	
	GG	47 (15.2)	12 (25.5)		16 (36.4)		7 (17.1)		8 (19.0)	
rs6198 (9β)	AA	227 (72.3)	53 (23.3)	.25	58 (27.4)	.49	26 (12.4)	.45	16 (7.7)	.02
	AG	75 (23.9)	22 (29.3)		22 (32.4)		12 (18.2)		12(18.2)	
	GG	12 (3.8)	1 (8.3)		2 (16.7)		2 (18.2)		0	
MR SNP										
rs2070951 (–2G/C)	GG	86 (28.5)	21 (24.4)	.85	22 (27.5)	.98	9 (11.3)	.79	10 (12.3)	.70
	GC	140 (46.4)	34 (24.3)		35 (26.7)		18 (14.1)		11 (8.7)	
	CC	76 (25.1)	21 (27.6)		20 (28.2)		10 (14.7)		7 (10.3)	
rs5522 (I180V)	AA	229 (75.1)	56 (24.5)	.75	62 (29.0)	.29	29 (13.8)	.63	22 (10.6)	.63
	AG+GG	76 (24.9)	20 (26.3)		16 (22.5)		8 (11.6)		6 (8.6)	

Associations are tested with Chi square tests and logistic regression analyses with age, sex and subtype of BD as covariates. P values of Chi square analyses are mentioned. All results are written as n(%).

Table 3. *Association of GR and MR gene polymorphisms and clinical characteristics in 326 BD patients*

	Geno-type	N (%)	Age of onset of depression		Age of onset of (hypo)mania		Number of (hypo) manic episodes		Number of depressive episodes	
			mean (SD)	p	mean (SD)	p	mean (95% CI)	p	mean (95% CI)	p
GR SNP										
rs10052957 (Tth*III*)	CC	155 (50.5)	24.5 (11.8)	.53	28.6 (11.0)	.74	6.4 (5.2-7.8)	.84	7.7 (6.4-9.3)	.76
	CT	118 (38.4)	28.8 (11.9)		29.5 (12.8)		6.2 (4.9-8.0)		7.8 (6.1-9.8)	
	TT	34 (11.1)	26.5 (11.8)		30.4 (12.3)		7.3 (4.1-12.5)		9.2 (5.7-14.4)	
rs6189 (ER22/23EK)	GG	291 (93.9)	26.1 (12.0)	.26	29.7 (12.1)	.004	6.4 (5.4-7.6)	.73	8.2 (7.0-9.6)	.05
	GA+AA	19(6.1)	22.8 (10.9)		21.9 (9.3)		5.8 (2.5-12.5)		4.3 (2.0-8.4)	
rs6195 (N363S)	AA	293 (93.6)	26.0 (11.9)	.71	29.4 (12.0)	.15	6.3 (5.4-7.5)	.76	8.0 (6.9-9.4)	.65
	AG	20(6.4)	24.9 (11.5)		25.6 (10.6)		6.9 (3.2-14.0)		6.4 (3.1-12.2)	
rs41423247 (B*c*l1)	CC	125 (40.3)	25.0 (11.9)	.24	27.4 (11.5)	.05	5.8 (4.7-7.2)	.62	7.8 (6.1-9.9)	.30
	CG	138 (44.5)	27.1 (11.9)		31.2 (11.7)		6.5 (5.2-8.1)		7.3 (6.0-8.9)	
	GG	47 (15.2)	23.0 (11.2)		27.7 (12.7)		7.2 (4.4-11.3)		10.1 (7.0-14.5)	
rs6198 (9β)	AA	227 (72.3)	25.0 (11.7)	.59	29.0 (11.7)	.96	6.5 (5.5-7.8)	.87	8.0 (6.8-9.4)	.71
	AG	75 (23.9)	27.0 (12.0)		29.3 (12.1)		6.1 (4.3-8.4)		7.2 (5.2-9.9)	
	GG	12 (3.8)	28.2 (13.3)		30.5 (15.0)		5.7 (2.2-13.0)		9.4 (3.9-21.2)	
MR SNP										
rs2070951 (–2G/C)	GG	86 (28.5)	26.4 (12.3)	.52	29.4 (12.5)	.54	5.4 (4.0-7.3)	.36	7.4 (5.6-9.8)	.75
	GC	140 (46.4)	24.7 (11.3)		28.3 (11.4)		6.5 (5.3-8.1)		8.5 (6.8-10.5)	
	CC	76 (25.1)	26.3 (12.2)		30.6 (11.9)		7.4 (5.3-10.2)		7.7 (5.7-10.1)	
rs5522 (I180V)	AA	229 (75.1)	25.5 (12.2)	.45	28.9 (12.0)	.52	6.1 (5.0-7.3)	.44	7.9 (7.7-9.5)	.71
	AG+GG	76 (24.9)	26.8 (11.5)		30.0 (11.9)		7.4 (5.3-10.3)		7.8 (5.6-10.7)	

Associations are tested with ANOVA and t-test analyses, and ANCOVA to adjust for age (number of episodes), sex and BD subtype (age of onset and number of episodes). P-values of t-tests and ANOVA's are mentioned. Number of episodes variables were both log transformed during analyses, and back transformed in this table.

Table 4. *Association of GR and MR gene haplotypes with clinical characteristics in BD 326 patients*

Variables	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 4	Haplotype 5	Haplotype 6	P value
No. of haplotypes; n (%)	289 (44.3)	155 (23.7)	91 (13.9)	78 (11.9)	21 (3.2)	19 (2.9)	
Age (yr); mean (SD)	47.3 (11.2)	47.9 (10.7)	50.0 (11.4)	49.1 (11.9)	46.2 (10.3)	46.7 (11.1)	.34
Male sex; n (%)	111 (38.7)	64 (41.6)	39 (42.9)	25 (32.5)	12 (57.1)	8 (42.1)	.40
Age of onset of depression; mean (SD)	25.6 (11.8)	25.8 (12.2)	26.0 (11.7)	28.2 (12.0)	22.7 (10.8)	25.5 (11.5)	.53
Age of onset of (hypo)mania; mean (SD)	28.8 (11.5)	30.1 (12.4)	30.1 (12.4)	31.1 (12.4)	21.7 (9.1)	26.0 (10.8)	.03
Number of (hypo) manic episodes; mean (95%CI)	6.1 (5.3-7.0)	6.4 (5.1-8.1)	7.1 (5.2-9.5)	5.6 (4.0-7.7)	7.7 (4.1-14.0)	6.8 (3.2-13.6)	.85
Number of depressive episodes; mean (95% CI)	7.5 (6.4-8.0)	7.6 (6.3-9.2)	9.0 (6.9-11.8)	8.8 (6.3-12.0)	4.7 (2.7-7.9)	7.0 (3.4-13.3)	.35
Current anxiety disorder; n (%)	67 (23.3)	37 (24.0)	27 (29.7)	23 (29.9)	1 (4.8)	5 (26.3)	.21
Seasonal pattern depression; n (%)	67 (25.5)	46 (32.2)	25 (30.1)	21 (29.2)	5 (26.3)	2 (10.5)	.39
Seasonal pattern mania; n (%)	33 (12.6)	19 (14.0)	12 (14.1)	11 (16.4)	5 (25.0)	2 (10.5)	.71
Seasonal pattern hypomania; n (%)	14 (5.4)	19 (14.0)**	10 (12.0)*	12 (17.1)**	0	1 (5.6)	.008

Associations of haplotypes are always in comparison with haplotype 1 (wildtype). Associations of haplotypes and dichotomous variables are tested with chi square tests and adjusted in logistic regression analyses for age, sex and subtype of BD. P-values are considered significant when <.01 to correct for multiple testing. Associations of haplotypes and continuous variables are tested with ANOVA. ANCOVA is applied to adjust for age, sex and subtype of BD. Post Hoc Dunnett's methods were used in post-hoc tests, correcting for multiple testing. Overall P values by ANOVA of chi-squared tests are presented.

** p<.01 **p<.001*

3.3 Glucocorticoid receptor (GR) genotype and bipolar phenotypic characteristics

Table 3 shows that the ER22/23EK carriers had slightly different characteristics with respect to age of onset. These carriers were significantly younger during the first (hypo) manic episode (crude 29.7 vs. 21.9, p<.004; however, after adjustment this was only a statistical trend with ages of respectively 29.5 vs. 23.1, p=.016). The onset of the first depressive episode also showed a tendency to a lower mean age in ER22/23EK carriers vs. noncarriers, but did not reach statistical significance.

Analyses on haplotype level (table 4) revealed that there was a tendency for a difference in age of onset of (hypo) mania among GR haplotypes (p=.03). Further analysis revealed a trend for an earlier age of onset of mania in subjects with GR haplotype 5 (crude 28.8 in GR haplotype 1 vs. 21.7 in GR haplotype 5, p=.06; after adjustment 29.0 vs. 22.2, p=.02). Number of episodes and age of onset of depressive episodes did not differ significantly among haplotypes.

Analyses at haplotype level showed that a seasonal pattern of hypomania was more frequent in carriers of the GR haplotypes 2 and 4 (Table 4). In GR haplotype 1 the frequency of seasonal hypomania was 5.4%. In GR haplotype 2 this was 14.0% (crude p=.004; after adjustment p=.007, OR= 2.83, 95%Confidence Interval [CI]:1.3-6.0), in GR haplotype 4 this was 17.1% (crude p=.001; after adjustment p=.005, OR=1.50, 95% CI: 1.13-1.98). GR haplotype 3 revealed a tendency for an association with seasonal pattern of hypomania with a frequency of 12.0% (crude p=.04; after adjustment p=.06, OR= 1.52, 95% CI: 0.98 - 2.34).

3.4 Mineralocorticoid receptor (MR) genotype and bipolar phenotypic characteristics

Table 2 and 3 present the associations between disease characteristics in relation to MR SNP analyses. No statistically significant associations between clinical disease characteristics of BD and any of the MR SNPs or haplotypes were found.

4. Discussion

In this study we investigated whether GR- en MR-gene polymorphisms were correlated with clinical manifestations of BD. We found evidence for a minor role of GR polymorphisms in influencing clinical aspects of BD. First, we found an association between seasonal patterns of hypomania and the *Bcl*I haplotype 2 and the T*thIII*I+9β

haplotype 4. This result was also found as a statistical trend association at the SNP level for the *Bcl*I SNP and the 9β SNP. Second, carriers of the ER22/23EK SNPs (located in haplotype 5) had an almost 8 years earlier onset of their first (hypo) manic episode than non carriers. Similar results were obtained in the haplotype analysis, showing that the T*thIII*I+9β+ER22/23EK haplotype 5 was associated with 7 years earlier age at the onset of the first manic episode. No evidence for a role of the MR in modifying clinical characteristics was found.

To our knowledge this is the first study showing a relationship between seasonal patterns of mood episodes in BD and genetic variation in the GR gene. Several studies have investigated the relation between seasonal changes of the HPA-axis responsivity in animals, healthy humans and depressed inpatients. Some studies found elevated morning cortisol levels in healthy humans during winter (30, 31). However, in a study with depressed patients a decreased sensitivity to Dexamethasone feedback of the HPA-axis was measured in autumn and spring, and an increased sensitivity in summer and winter (32). In mice the highest GR expression was found in January (33), also illustrating an increased GC sensitivity during winter. In parallel, mice studies showed which higher GR expression in the hippocampus of mice during short days and a faster return to basal cortisol levels after restraint (34). One of the mechanisms leading to seasonal changes in cortisol sensitivity is thought to be the direct effect of change in day lengths on the activity of the suprachiasmatic nucleus (SCN) in the hypothalamus (35, 36). This is thought to be mediated by reduced expression of clock genes in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus and the hippocampus, which was found in European hamsters (37). In order to understand the relation between seasonal changes of the HPA-axis and mood episodes in BD patients with GR SNPs, it is important to emphasize that these SNPs alter HPA-axis sensitivity. The *Bcl*I and the N363S SNP are known to increase Glucocorticoid sensitivity (38-40). The 9β and the ER22/23EK SNP are both associated with a mild resistance for GCs (41, 42). We hypothesized that these genetic variants in corticosteroid receptor genes confer susceptibility for the precipitation of psychiatric disorders under less favorable conditions (43). In the context of seasonal changes these SNPs could bring the precisely regulated HPA-axis off balance when adjusting for seasonal changes. This gene-variant – environment interaction might well play a role in patients with BD where seasonal changes of daylight are known to influence mood (44), which could be worsened by the described SNPs. We found associations between haplotypes 2 (*Bcl*I) and 4 (T*thIII*I+9β) only with seasonal patterns of hypomanic episodes, and not with manic episodes. This is in line with the finding of a 10-year prospective study among 302 bipolar patients, revealing more seasonal episodes in patients with BD2 (2). Besides, when looking at seasonal patterns in mood episodes, irrespective of subtype of bipolar

disorder, a clear association with depression as well as with hypomania was found, but not with mania.

The finding that carriers of the ER22/23EK SNPs had an earlier onset of the first (hypo) manic episode has not been reported earlier. Earlier onset may be related to a worse outcome (27, 45). The finding that carriers of the ER22/23EK SNP were younger when the first symptoms of BD appeared and may consequently have had a higher risk on a poorer outcome with more mood episodes, is in accordance with previous studies demonstrating that the ER22/23EK variant correlated to an increased susceptibility for dysregulation of mood (17, 46-49). On the other hand these studies indicate a more beneficial outcome for carriers of these SNPs (or this haplotype). In line with the hypothesis of a beneficial outcome, we found a statistical trend towards less prevalent anxiety disorders in patients with the ER22/23EK variant (5.3% vs. 25.1% in non carriers). Previously, Russcher et al showed that the transcriptional activity of the GR in ER22/23EK carriers is decreased because more of the less transcriptionally active GR-A isoform is formed, which seems to be caused by altered secondary mRNA structure (50). The subtle resistance for GCs in ER22/23EK carriers may result in compensatory higher cortisol levels, which may have tissue-specific increased effects in the limbic system yielding mood disturbances. Another explanation we could speculate on is that the insensitive GR signaling due to the ER22/23EK variant leads to intracellular deficit of cortisol in the limbic system yielding an increased vulnerability for mood disturbance, and at the same time a protective effect on the long-term with respect to other brain areas related to anxiety. However, further research is needed to confirm these speculations and elucidate the neurobiological pathways.

The MR is known to be involved in the regulation of both low levels of cortisol and the reactivity of the stress-response following a challenge (13, 15). There is some evidence for an association between MR gene polymorphisms and social stress situations in adults. In one study (24) an association between depressive symptoms and the V-allele has been found, and another study provides evidence for an association between MR-SNPs and neuroticism (25). No such associations were found in adolescents (51). Since we found no evidence for a role of the MR in modifying number of episodes, age of onset and co morbidity in BD this might indicate that environmental influences predominantly interact with gene-variants of the GR but less so with the MR.

There are several limitations in this study. First, the ER22/23EK SNPs were not in Hardy Weinberg equilibrium, which may be explained as a chance finding in combination with a low allele frequency. Yet, it is unlikely that this deviation influenced the outcomes of the analyses. A second limitation of this study is the absence of measurements of serum cortisol levels and other endocrinological clinical parameters. Serum cortisol could

provide information about the potential mediating effects of cortisol on the relationship between GR and MR SNPs and clinical characteristics in BD patients. However, serum cortisol measurements may fail to adequately reflect cortisol's true impact because of the circadian rhythm of cortisol levels and the pulsatile way cortisol is secreted. Also, large daily variations due to e.g. acute stress or infection are also an important limitation of measuring cortisol levels. Using information from genetic variations known to be associated with altered cortisol sensitivity may better contribute to understanding the relationship between glucocorticoids and BD. Third, we used a relatively small sample size for a genetic association study, although we used functionally characterized genetic variants. Moreover, we were not able to perform a replication study in a different sample. Fourth, in this study no data were obtained with regard to premorbid functioning, known to be an important predictor for outcome of first episode psychosis. However, information on premorbid functioning is often based on patients recalling and difficult to assess in an unbiased way. Fifth, our study design was cross-sectional with clinical data gathered retrospectively, leading to potential recall bias. This could have affected the reliability of the retrospectively collected data on age of onset. Patients were questioned about their first illness symptoms, which hamper a sharp differentiation between hypomania and mania symptoms. Finally, BD is genetically a very complex disease. Besides HPA-axis related factors, numerous other environmental and genetic factors also influence clinical BD characteristics. Therefore, findings on number of episodes and seasonality need to be further explored and replicated in larger and prospective studies. Future prospective studies should include clinical characteristics like cognition and physical health. The ultimate goal is to obtain more insight in risk factors for poor outcome and subsequently develop individualized therapeutic interventions.

We conclude that GR SNPs affect several clinical manifestations of BD. Seasonal mood episodes are likely to develop in BD patients with altered HPA-axis regulation, at least partly caused by genetic variation in the GR. Together with natural changes of GC sensitivity throughout the year this could increase vulnerability for mood episodes.

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