Cortisol exposure, cognition and clinical course of bipolar disorder

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4. Functional Polymorphism of the Glucocorticoid Receptor Gene associates with mania and hypomania in Bipolar Disorder

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Abstract

Objectives

In affective disorders, dysregulation of the hypothalamus-pituitary-adrenal (HPA)-axis is a frequently observed phenomenon. Subtle changes in Glucocorticoid Receptor (GR) functioning caused by polymorphisms of the GR gene (*NR3C1*) may be at the base of the altered reaction of the HPA-axis to stress and subsequently related to the development and course of affective disorders. The aim of our study is to evaluate associations between GR gene polymorphisms and bipolar disorder (BD).

Methods

In this study 245 patients with BD were interviewed to confirm diagnosis and BD subtype. Data on medication use and sociodemographic details were also collected. The control group consisted of 532 healthy blood donors, of which data on sex and age were collected. To perform genotyping blood was collected from all patients and healthy controls.

Results

A trend was found for a protective effect of the exon 9β-polymorphism ($p=0.14$) and the *TthIII*I-polymorphism ($p<0.05$) on the manifestation of the disease. These effects were significantly influenced by male gender for both polymorphisms. Patients with BD and the A/G variant in exon 9β had significantly less manic and hypomanic episodes than noncarriers ($p<0.05$).

No further associations were found with the other investigated GR gene polymorphisms and BD. These findings were not corrected for multiple comparisons.

Conclusions

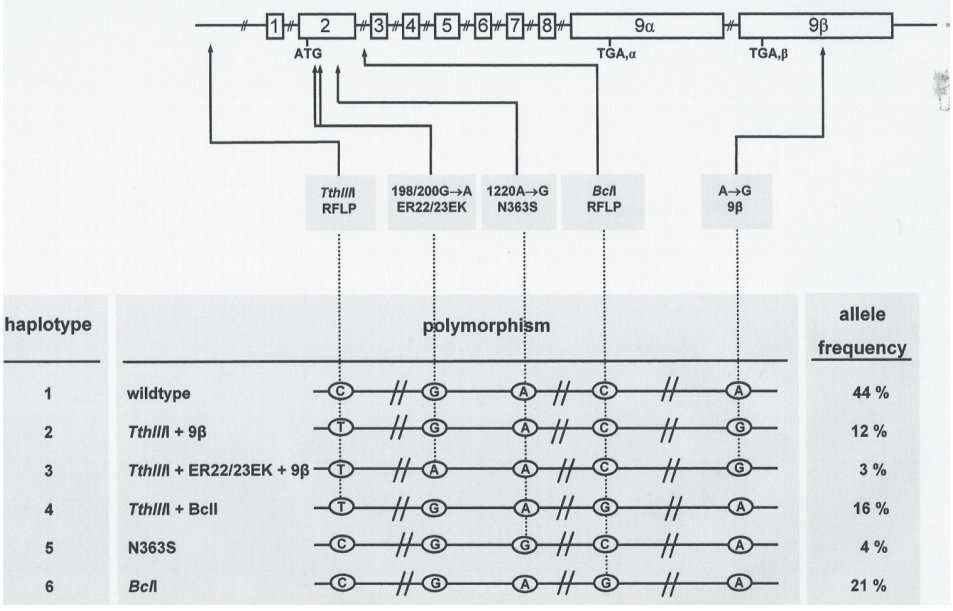
We conclude that the exon 9β-polymorphism and the *TthIII*I-polymorphism of the GR gene may be associated with a protective effect on the clinical manifestation and course in patients with BD. Furthermore no associations were found between the other studied GR gene polymorphisms and this disease.

Introduction

Bipolar Disorder (BD) is a common illness, with a lifetime prevalence of 1.9 % (1) to 2.4% (2). It is also associated with a high lifetime prevalence (82.8 %) of co morbid mental disorders, specifically anxiety disorders (31.9%) (3). The course of the disease is highly variable and unpredictable. As it is known that there is a dysregulation of the stress response in these patients, it is important to evaluate the influence of this dysregulation on the course of the disease.

Dysregulation of the stress response, or specifically dysregulation of the hypothalamus-pituitary-adrenal (HPA)-axis is known to occur in several psychiatric disorders, including BD (4-10). Cortisol exerts its effects through the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Recently, several polymorphisms of the GR and MR have been demonstrated to influence the response of the HPA-axis to stress (11-14). Figure 1 shows a schematic overview of the GR-gene *NR3C1* with the functional polymorphisms, as well as their haplotypes.

Figure 1 Schematically overview of the GR-gene, the different Single Nucleotide Polymorphisms and haplotypes.



Haplotypes 2-6 have been described as associated with a change in regulation of the HPA-axis. In two recent studies, haplotype 3 (*Tth*III*I* + 9β + ER22/23EK) was found to be associated with a higher risk of major depressive disorder (15, 16) and a faster response to antidepressant medication (15). In addition, the *Bcl*I-polymorphism (haplotypes 4 and 6) was more prevalent in depressed patients (15). In a recent study, carriers of the *Bcl*I-polymorphism with a major depressive episode had higher AdrenoCorticoTropic Hormone (ACTH) levels and a tendency to worse treatment outcome (17).

In this study, the prevalence of these polymorphisms in the GR-gene of patients with BD will be assessed, as well as their relationship with the course and severity of BD.

Patients and Methods

Subjects

This study is a cross-sectional, explorative case-control study of outpatients with BD. Patients with BD type 1, BD type 2 and BD Not Otherwise Specified (NOS), according to DSM-IV criteria were included. The design of the study was approved by the independent Dutch national medical ethics committee. All patients treated in the outpatient Clinic for Mood Disorders in The Hague were invited to participate. All 412 patients being treated for BD by the Outpatient Clinic for Mood Disorders in The Hague (Netherlands) were invited to participate in the study, either by letter or directly by their treating physician. After written informed consent was obtained, 245 patients were enrolled. To assess the diagnosis and co morbidity according to the DSM-IV criteria, patients were interviewed by trained research assistants using the Mini International Neuropsychiatric Interview Plus (18). Sociodemographic and ethnic data were collected, and the Clinical Questionnaire (CQBP-C) (Dutch translation Akkerhuis, Groenesteyn, Nolen 1997) was used to specify subtypes of BD and its course over time.

Blood samples from 532 healthy control subjects were randomly collected from the Rotterdam blood donation bank. Only the gender and age of these subjects are known.

Genotyping

From each patient, 40 ml of blood were collected in EDTA tubes and DNA was extracted from fresh blood using the Puregene whole blood DNA-extraction kit (Gentra Systems Inc; MN). Allelic discrimination was performed to genotype the subjects, using TaqMan Universal PCR master mix, and custom designed primers and probes (Applied Biosystems, Nieuwerkerk aan den IJssel, Netherlands, see also Table 1 and an Applied Biosystems

7900 HT Sequence Detection System as previously described (13). Reaction components and amplification parameters were based on the manufacturer's instructions. The genotypes were reanalyzed for all heterozygous and homozygous carriers of the single nucleotide polymorphisms (SNPs) and identical genotypes were identified. Both groups (controls and patients) were found to be in Hardy-Weinberg equilibrium with respect to all five polymorphisms. The accession number of the GR gene is NM_000176; the various polymorphisms have the following accession numbers: *TthIII*I - rs10052957; ER22/23EK - rs6189 and rs6190; N363S: rs6195; *Bcl*I - rs41423247; 9β - rs6198.

Table 1. *Sequences of primers and probes used for genotyping.*

Polymorphism	Primers	
Tth	Fw	5'-GGAGTGGGACATAAAGCTATGACAA-3'
	Rev	5'-GCAGAGGTGGAAATGAAGGTGAT-3'
N363S	Fw	5'-CAACAGCAGGATCAGAAGCCTAT- 3'
	Rev	5'-CCCAGAGAAGTCAAGTTGTCATCTC- 3'
ER22/23EK	Fw	5 '-TCCAAAGAATCATTAACTCCTGGTAGA- 3'
	Rev	5'-GCTCCTCCTCTTAGGGTTTTATAGAAG- 3'
*Bcl*I	Fw	5'-GCTCACAGGGTTCTTGCCATA- 3'
	Rev	5'-TTGCACCATGTTGACACCAAT- 3'
9-beta	Fw	5'-TCAGACTGTAAAACCTTGTGTGGAA-3'
	Rev	5'-CCAATTCGGTACAAATGTGTGGTT-3'

Polymorphism		Probes
Tth	Wt	5'-FAM-TTCAGACTCAATCAAGG-3'
	Mu	5'-VIC-TATTCAGACTCAGTCAAGG-3'
N363S	Wt	5'-FAM-CCTATTCCAAT<u></u>TTTCGGAACCAACGG- 3'
	Mu	5'-VIC- CCTATTCCAA<u>C</u>TTTCGGAACCAACGG - 3'
ER22/23EK	Wt	5'-FAM-ACATCTCCC<u>C</u>T<u>C</u>TCCTGAGCAAGC-3'
	Mu	5'-VIC-ACATCTCCC<u>TTT</u>TCCTGAGCAAGC-3 '
*Bcl*I	Wt	5'-FAM-TCTGCTGAT<u>C</u>AATCT -3'
	Mu	5'-VIC-TCTGCTGAT<u>G</u>AATCT - 3'
9-Beta	Wt	5'-FAM-TTTATTTTTTCGTTTAAATTT-3'
	Mu	5'-VIC-CTTTTATTTTTTCATTTAAATTT-3'

Fw=forward, Rev=reverse, Wt=wild type, Mu=mutant

Statistical analysis

Data were analyzed using SPSS (release 12.0.1 for Windows (SPSS, Chicago, IL)). Analyses for binary outcomes (case-control and dichotomous response variables) were performed with logistic regression analysis. The patient group and the control group significantly differed in age and gender. Therefore all analyses were corrected for age and gender.

First, frequencies of polymorphisms were analyzed in the patient and the control group. Because only age, gender and genotype were known from the control group, in the logistic regression analysis only these variables were added as independent explanatory variables.

Second, clinical data within the patient group were analyzed in relation to genotypes. Phenotyping was focused on current situation, and little was known about course and severity of the disease. Therefore, number of episodes was chosen as an indicator of severity of the disease. Groups concerning number of depressive or (hypo-) manic episodes were chosen based on the median number of episodes (see Table 2).

To reduce the problem of multiple testing, only the logistic regression analysis was performed for each genotype using frequency and number of episodes as dependent variables. As this is an explorative, hypothesis generating study, data mining is not completely avoidable. In this study the statistical power was not big enough to apply the Bonferroni's correction or the split sample method.

Haplotypes were not included in the analyses as the number of patients was not sufficient.

Results

Of the 245 patients enrolled in the study, genotyping failed in three patients and one patient did not have BD as defined by the MINI and the CQBP. The baseline characteristics of the remaining 241 patients and the control group are described in Table 2.

The *TthIII*I polymorphism was only analyzed in a subgroup of 320 controls. In 23 patients data on number of (hypo) manic episodes were missing.

Table 2 *Baseline Characteristics*

	Bipolar Patients	Controls
Number	241	532
Age (years)	47.8 ± 10.8	42.7 ± 12.2
Gender (% male)	102 (42%)	285 (54%)
Ethnicity	229 (95%) Caucasian	
Bipolar Disorder type 1	198 (82.2%)	
Bipolar Disorder type 2	41 (17%)	
Bipolar Disorder NOS	2 (0.8%)	
Age of onset (years)		
First depressive episode	25 ± 11,6	
First (hypo)manic episode	29 ± 11,7	
Depressive episodes		
Mean number	15 ± 21,2	
Median	6	
Manic episodes		
Mean number	12 ± 19,9	
Median	5	
Inter-episode functioning	142 (58,9%) well functioning	
	61 (25,4 %) some problems, most of the time well functioning	
	16 (6,6%) severely disturbed	
	22 (9,1%) no difference between episodes	
Use of medication	203 (84%) Lithium	
	31 (13%) Valproaat	
	8 (3%) Carbamazepine	
	11 (5%) Lamotrigine	
	71 (30%) Antidepressants	
Comorbidity	108 (47%) ≥1 Comorbid disorders	
	91 (39%) Anxiety disorder	
	10 (4%) Somatoform disorder	
	9 (4%) Pain disorder	
	6 (3%) Boulimia nervosa	
	5 (2%) Drug dependency	
	12 (5%) Alcohol dependency	
	6 (3%) ADHD	
	15 (11%) of all women have premenstrual dysphoria	

Comparison of frequencies of GR polymorphisms

In subjects of the patient group, a significantly lower frequency of heterozygous *TthIII*I carriers compared to the control group was found: 36.9% vs. 45.0% (p= 0.03, OR 0.51, 95% CI: 0.27-0.95). We found no differences in frequency of homozygous *TthIII*I variation, nor any other differences in genotype frequency between healthy controls and the group of bipolar patients (Table 3). Age had no significant effect: OR 1.0, 95%CI 0.98-1.01. Male gender appeared to have a significant effect in this analysis: OR 0.40, 95%CI 0.28-0.57, p<0.0001.

We found a non-significant trend for a lower frequency of the A/G or G/G variation in exon 9β in the patient group vs. the control group (respectively 24.5% vs. 31.0%, OR 0.76, 95%CI= 0.5-1.0; p=0.14, corrected for age and gender). Both age and gender had significant influences (for age: OR 1.04, 95%CI 1.02-1.05, p=0.000; for male gender: OR 0.52, 95%CI 0.37-0.72, p<0.0001).

Table 3 *Frequencies of five polymorphisms of the Glucocorticoid Receptor Gene in healthy control subjects and bipolar patients, and frequencies in patients with 0-5 hypomanic and manic episodes versus patients with >5 manic episodes; number (percentage)*

*Legend: *p= 0.03, OR 0.51 (95% CI: 0.27-0.95); ** p= 0.14, OR 0.76 (95% CI: 0.53-1.09)*

#p= 0.04, OR= 0.5 (95% CI: 0.27-0.98); ## p = 0.02, OR 0.46 (95% CI: 0.23-0.91)

	Healthy controls	**Bipolar patients**		
Polymorphism			with 0-5 manic and hypomanic episodes	>5 manic and hypomanic episodes
*TthIII*I				
CC	150 (46.9%)	126 (52.3%)	61 (51.3%)	53 (53.5%)
CT	144 (45.0%)	**89 (36.9%)***	46 (38.7%)	34 (34.3%)
TT	26 (8.1%)	26 (10.8%)	12 (10.1%)	12 (12.1%)
CT + TT	170 (53.1%)	115 (47.7%)	58 (48.7%)	46 (46.5%)
ER22/23EK				
GG	495 (93.0%)	227 (94.2%)	110 (92.4%)	94 (94.9%)
GA	36 (6.8%)	12 (5.0%)	8 (6.7%)	4 (4.1%)
AA	1 (0.2%)	2 (0.9%)	1 (0.9 %)	1 (1.0%)
GA + AA	37 (7.0%)	14 (5.8%)	9 (7.6%)	5 (5.1%)
N363S				
AA	493 (92.7%)	228 (94.6%)	111 (93.3%)	95 (96.0%)
AG	39 (7.3%)	13 (5.4%)	8 (6.7%)	4 (4.0%)
GG	0	0	0	0
AG + GG	39 (7.3%)	13 (5.4%)	8 (6.7%)	4 (4.0%)
*Bcl*I				
CC	199 (37.4%)	97 (40.2%)	53 (44.5%)	36 (36.4%)
CG	255 (47.9%)	110 (45.6%)	52 (43.7%)	48 (48.5%)
GG	78 (14.7%)	34 (14.1%)	14 (11.8%)	15 (15.2%)
CG + GG	333 (62.6%)	144 (59.8%)	66 (55.5%)	63 (63.6%)
9ß				
AA	367 (69.0%)	182 (75.5%)	82 (68.9%)	80 (80.8%)
AG	147 (27.6%)	51 (21.2%)	33 (27.7%)	**15 (15.2%)##**
GG	18 (3.4%)	8 (3.3%)	4 (3.4%)	4 (4.0%)
AG + GG	165 (31.0%)	**59 (24.5%)****	37 (31.1%)	**19 (19.2%)#**

Association of the GR polymorphisms with number of depressive and manic episodes

We further explored the association between number of mood episodes and the GR polymorphisms (Table 3). The number of episodes was dichotomously analyzed in four groups, based on the median number of episodes (see Table 2): the number of depressive episodes is 0 to 6 or more than 6, and the number of manic and hypomanic episodes is 0 to 5 or more than 5. Table 3 shows the frequencies of the GR polymorphisms in relation to number of manic and hypomanic episodes.

The most important finding is the difference in frequency of the exon 9β polymorphism between the group of patients with more and the group with less than 5 manic and hypo manic episodes (respectively 19.2% and 31.1%; p= 0.04, OR 0.5, 95%CI = 0.27-0.98). This difference is even more significant for heterozygous carriers (respectively15.2% and 27.7%; p=0.02, OR 0.46, 95%CI: 0.23-0.91). In both analyses age and gender had no significant effects. Additional analyses using nearby cut- off points yielded similar results. With respect to the number of depressive episodes, no significant differences between the group of patients with 0-6 episodes and the group of patients with >6 episodes, were found in the frequency of exon 9β-carriers.

None of the other comparisons (see Table 3) reached statistical significance.

Discussion

The major findings in this study are the lower prevalences of the *TthIII*I and exon 9β polymorphisms in the patient group compared to controls. These findings are consistent, since the 9β polymorphism is always present in combination with the *TthIII*I polymorphism, as is shown in Figure 1. Furthermore, those patients who carry the exon 9β polymorphism have significantly fewer manic episodes. This may indicate that the presence of the exon 9β-polymorphism has a (subtle) protective effect on both the prevalence and the clinical course of the disease. Clearly, these findings need replication to confirm the observations.

A possible limitation of the chosen cut-off points in number of episodes (mania more or less than 5, and depression more or less than 6) is that this is not based on clinical guidelines, but only on median number of episodes in our study population. However, other cut-off points in number of manic and hypo manic episodes yielded similar results. In future research, the course or severity of the disease will have to be carefully defined and prospectively followed to test these results.

We found no differences in the frequencies of the other GR gene polymorphisms between the controls and the patient group. In a recent study by Van Rossum (15) a higher prevalence of *Bcl*I and ER22/23EK polymorphisms was found in 495 hospitalized, severely depressed patients (13.2% with BD, 86.8% unipolar depressive disorder). In our study, patients differed considerably with respect to the severity of their illness, and none of the patients suffered from unipolar depressive disorder. Furthermore, the number of patients in our study was smaller, which potentially resulted in a lack of statistical power to detect minor differences.

This negative finding seems to indicate that these polymorphisms have no major effect on the etiology of the illness.

This study was the first to explore the theoretically relevant association between BD and genetically determined stress vulnerability as reflected by different GR polymorphisms. A limitation of this study is that the findings are not replicated yet in another population of patients with BD. It is clear that further research is needed to confirm these results.

Both findings in this study (the lower frequency of the *Tth*III*I* and exon 9β polymorphisms in the patient group, and the association of the G/A-polymorphism in exon 9β with less manic episodes) are in line with the modest conclusion that this haplotype may be associated with a protective effect on the illness. We found significant associations, but we cannot rule out that these associations are explained by a type I error.

The question remains, what could be the possible cause of the negative relationship between BD and the G/A-polymorphism in exon 9β of the GR gene. This polymorphism has a stabilizing effect on the mRNA levels of the GR-β isoform (19). This could result in a rise of GR-β levels, leading to a relative resistance to glucocorticoids since GR-b has been shown to exert a dominant negative inhibition of the active GR-α isoform. This polymorphism is also associated with the functioning of the immune system (19,20). This seems important, as several abnormalities in the functioning of the immune system are present in patients with BD. These include elevated IL-6 production, increased risk of autoimmune phenomena (such as autoimmune thyreoiditis, and raised thyroperoxidase auto-antibodies) and abnormalities in monocyte differentiation, indicating a dysregulation of the immune system (21, 22). However, the mechanisms for these associations among GR-β expression, changes in the functioning of the HPA-axis, and abnormalities in the immune system are largely unknown.

As mentioned in the introduction, there is ample evidence of dysregulation of the HPA-axis in patients with BD, possibly due to GR dysfunctioning. Our study found evidence that two commonly occurring GR gene polymorphisms are associated with the clinical manifestation and the course of bipolar disorder. Further research is needed to elucidate

the influence of stress vulnerability as revealed by GR and MR gene polymorphisms on the prognosis of BD. This might offer avenues for the development of new therapeutic interventions targeting the stress system.

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