# Glucocorticoid receptor polymorphism is associated with lithium response in bipolar patients

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## Abstract

**OBJECTIVES:** Glucocorticoid receptor has been considered in the pathogenesis of bipolar disorder as it is an important regulator of the circadian rhythm and HPA negative feedback. As glucocorticoid receptor may be possibly related to lithium action we aimed to investigate variation in the GR gene in association with response to lithium treatment in Polish population of bipolar patients.
**METHODS:** We analyzed 115 bipolar patients treated with lithium carbonate for 5–27 years. Thirty patients were identified as excellent lithium responders (ER), 58 patients as partial responders (PR), and 27 patients were non-responders. Genotypes of eight analyzed polymorphisms of GR gene (rs10052957, rs6196, rs6198, rs6191, rs258813, rs33388, rs6195, rs41423247) were established by TaqMan SNP Genotyping Assays. Statistical analysis was done with Statistica version 9.0. Linkage disequilibrium analysis was performed in Haploview v. 4.1.
**RESULTS:** We have found significant differences in allele frequencies for BclI polymorphism between patients with different lithium response with C allele associated with excellent lithium response. For the other GR polymorphisms any significant association with different lithium response was found. We observed a strong linkage disequilibrium of five GR polymorphisms (rs6198, rs6191, rs6196, rs258813, rs33388), with TAAGA haplotype more prevalent in the group of partial- and non-responders to lithium.
**CONCLUSION:** The GR gene variation seems to be involved in the response to lithium treatment in our group of bipolar patients.

ORIGINAL ARTICLE

## INTRODUCTION

Bipolar disorder is characterized by abnormal function of hypothalamic-pituitary-adrenal (HPA) axis. Pituitary gland volume is decreased in bipolar patients as compared to controls and this finding is consistent with pituitary hypoactivity in response to HPA stimulation in patients with bipolar disorder (Sassi *et al.* 2001). Moreover, several authors reported abnormalities in urinary and cerebrospinal fluid cortisol levels (see Daban *et al.* 2005 for a review). Another characteristic is a loss of glucocorticoid sensitivity associated with impaired response in the dexamethasone (dex) suppression test and combined dex/CRH test (Heuser *et al.* 1994). In a study by Watson *et al.* it was suggested that HPA axis function is abnormal in either remitted and non-remitted bipolar patients (Watson *et al.* 2004), whereas in the other study (Rybakowski and Twardowska 1999) it was demonstrated that a dysregulation of HPA axis, as measured by DEX/CRH test, was more marked in patients with depression in the course of bipolar illness as compared to patients with unipolar depression. Moreover, HPA axis dysfunction was also suggested to play critical role in the switch from mania to depression in most of ultra-rapid cycling bipolar patients (Juckel *et al.* 2000).

Glucocorticoids (GCs) mediate their effect via intracellular glucocorticoid receptor alpha (GRα) and beta (GRβ) isoforms, both present in hippocampus and hypothalamus as target sites of HPA feedback regulation. Glucocorticoid receptor has been considered in the pathogenesis of bipolar disorder as it is an important regulator of the circadian rhythm and HPA negative feedback. Studies on GR expression in peripheral mononuclear blood cells and brain of bipolar patients remain controversial, showing either no change or decrease in GR levels (Pariante and Miller 2001; Webster *et al.* 2002). Study by Spiliotaki *et al.* (Spiliotaki *et al.* 2006) showed altered intracellular signaling cascade of glucocorticoid receptor in lymphocytes of bipolar patients.

The gene encoding the *GR* is one of the primary candidates for the vulnerability for stress related disorders such as bipolar disorder. Glucocorticoid receptor gene variants were previously found to be associated with clinical manifestation and the course of disease in bipolar patients demonstrating protective effect of 2 SNPs (rs10052957 and rs6198) in regard to number of manic and hypomanic episodes in the course of bipolar disorder (Spijker *et al.* 2009).

In the treatment and prophylaxis of bipolar disorder, lithium has been widely used for several decades. However, the therapeutic mechanism of lithium action in the treatment of bipolar disorder is still not well understood. Preclinical studies suggest that lithium may modulate glucocorticoid receptor expression in different brain areas: hippocampus and paraventricular nucleus of the hypothalamus (Semba *et al.* 2000). Recently, BAG-1, a co-chaperone protein involved in GR function, was one of the several genes identified in microarray studies that expression was upregulated by chronic lithium administration (Zhou *et al.* 2005) suggesting that GR-related biological pathways may be involved in the lithium action. In a study by Bschor *et al.* (Bschor *et al.* 2002) it was found that depressive patients not responding to antidepressants under lithium augmentation showed elevated response in ACTH and cortisol response in DEX/CRH test.

Based on the possible involvement of *GR* gene variation in lithium treatment response modification, we aimed to analyze 8 SNPs in the *GR* gene in association with different outcomes of lithium prophylaxis in 115 bipolar patients. To our knowledge, this is the first study of association analysis of *GR* gene with the response to lithium treatment.

## METHODS

*Patients*

In our analysis, 115 patients with bipolar affective disorder (42 males and 73 females) aged 30–77 years (mean age 52.4 years) recruited from the Outpatient Lithium Clinic at the Department of Psychiatry in Poznan University of Medical Sciences were included. Consensus diagnosis by at least two psychiatrists was made for each patient, according to DSM-IV and ICD-10 criteria (SCID) (First 1996). The patients have been treated with lithium carbonate for at least five years (5–27 years, mean 15 years). The patients have been attending the same outpatient clinic for the entire period of lithium administration. Serum concentration of lithium has been maintained in the range between 0.5–0.8 mmol/L as recommended in European guidelines (Ulrich *et al.* 2007). The course of illness was assessed retrospectively, based on the analysis of medical outpatient charts, inpatient records and semi-structured reviews as described previously (Rybakowski *et al.* 2005a; Rybakowski *et al.* 2005b).

The efficacy of lithium treatment was assessed according to the following criteria: excellent lithium responders (ER) had no affective episodes on lithium; partial lithium responders (PR) showed 50% reduction in the episode index (number of episodes per year to pre-lithium period); lithium non-responders (NR) showed < 50% reduction, no change or worsening in the episode index. In all excellent responders lithium has been given as monotherapy. Among remaining patients, antidepressant, neuroleptic and other mood stabilizing drugs were temporarily added for the treatment of depressive, manic or mixed episodes and for improving prophylactic lithium effect. None of the patients was treated with electroconvulsive therapy at the time of study. Also, there were no pregnancies in patients studied during lithium treatment.

All patients were recruited from the ethnically homogenous Wielkopolska region of Poland. After complete description of the study to the subjects, written informed consent was obtained. The study was

approved by the Local Bioethics Committee. Study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

*Genotyping*

The DNA was extracted from 10 ml of EDTA anticoagulated whole blood using the salting out method (Miller *et al.* 1988).

The SNP selection included the following criteria: potential functionality, high frequency (MAF >0.05), indication as tag SNP in HapMap or previously reported associations for psychiatric disorders (both positive and negative findings). SNPs chosen cover the whole gene region, both coding regions of known functionality as well as non-coding regions (introns, UTRs) possible to affect gene regulation.

The eight polymorphisms were genotyped with use of TaqMan Genotyping Master Mix and TaqMan SNP Genotyping assays (Applied Biosystems). The list of SNPs analyzed and the ID numbers of TaqMan assays were shown in Table 1. All the assays were validated and predesigned except for two polymorphisms (rs6195 and rs41423247) for which custom assays were designed. Reaction components and amplification parameters were based on manufacturer's instructions. The amplification for TaqMan SNP genotyping assay plates was done in ABI PRISM® 7900HT Sequence Detection System. Data acquisition and analysis was performed using the allelic discrimination analysis module in SDS v2.1 software (Applied Biosystems).

For each reaction plate genomic control DNA samples and non-template controls (water) were included. The control of TaqMan SNP genotyping assay was also performed (25% of randomly chosen samples from both groups) to check for genotyping accuracy and identical genotypes were identified in all repeated samples. The genotyping was performed without knowing the clinical status of the subjects.

*Statistical analysis*

The Pearson's chi-square ($\chi^2$) test and Fisher's exact test were used to test differences in the genotypic and allelic (respectively) distribution between the groups of patients with different lithium response. Calculations were performed using the statistical package Statistica version 7.1. For polymorphisms containing < 5 observations per cell we performed Fisher-Freeman-Halton exact test with use of StatsDirect statistical software v.2.6.2 (trial). Correction for multiple testing was also performed. Odds ratios were calculated using demo of GraphPad InStat 3 software. Linkage disequilibrium (LD) between SNPs polymorphisms of *NR3C1* gene was examined by pair-wise comparisons of $r^2$ and D using Haploview version 4.1 (Barrett *et al.* 2005).

**Tab. 1.** Description of *NR3C1* polymorphisms analyzed in this study.

| SNP ID | Substitution | Custom name | TaqMan assay ID |
|---|---|---|---|
| rs10052957 | A/G | Tth111I | C__30158011_10 |
| rs6196 | A/G | Asn766Asn | C__1046361_10 |
| rs6198 | C/T | GR9β | C__8951023_10 |
| rs6191 | A/C | – | C__3234245_20 |
| rs258813 | A/G | – | C__1046357 |
| rs33388 | A/T | – | C__1046426_10 |
| rs6195 | A/G | N363S | Custom assay |
| rs41423247 | C/G | BclI | Custom assay |

## RESULTS

In our group, 24 patients (23.7%) were classified as excellent responders, 51 patients (50.5%) as partial responders and 26 patients (25.7%) as non-responders to lithium treatment. A clinical characteristic of the group of patients was presented in Table 2.

Age at onset of bipolar affective disorder, duration of illness before treatment and during lithium prophylaxis and number of affective episodes before lithium treatment were not significantly different between the subgroups of patients with different treatment outcome.

Genotype distributions for all studied polymorphisms in the *GR* gene were in concordance with Hardy-Weinberg equilibrium law in all subgroups of patients treated with lithium ($p$>0.05) except the group of partial responders for rs6196 polymorphism.

**Tab. 2.** Clinical description of the patients.

| | Total n=115 | ER n=30 | PR n=58 | NR n=27 |
|---|---|---|---|---|
| Age years [mean±SD] | 52.4±11.9 | 56.0±12.6 | 50.7±11.8 | 52.4±11.8 |
| Gender [M:F] | 42 : 73 | 12 : 18 | 20 : 38 | 10 : 17 |
| Family history of psychiatric illness N (%) | 46 (40.0%) | 13 (43.3%) | 27 (46.6%) | 6 (22.2%) |
| Age at onset - years [mean±SD] | 29.6±9.3 | 29.6±8.8 | 29.5±9.4 | 30.1±9.9 |
| Duration of illness before lithium – years [mean±SD] | 7.4±7.4 | 9.7±9.6 | 5.6±5.9 | 8.7±6.0 |
| Duration of lithium treatment – years [mean±SD] | 14.6±7.3 | 14.0±7.1 | 15.3±7.9 | 13.8±5.8 |
| Affective episodes before lithium N [mean±SD] | 6.2±4.1 | 7.0±3.6 | 6.0±4.5 | 5.8±3.9 |
| Affective episodes on lithium N [mean±SD] | 3.3±3.9 | 0 | 3.5±2.7 | 8.2±4.8 |

We have not observed significant differences in genotypic distribution of *GR* polymorphism between different groups of lithium responders. In allelic distribution, we found an association of G allele of rs41423247 (previously known as BclI) polymorphism and lithium response (Table 3). When we pooled together patients with partial or no response versus patients with excellent response to lithium, we observed an association of the same polymorphism for allelic distribution with worse lithium response (Table 4).

In linkage disequilibrium analysis one haplotype block of five SNPs (rs6198, rs6191, rs6196, rs258813, rs33388, respectively) was created in Haploview analyzing patients with excellent lithium response versus patients with partial and poor response (Figure 1).

We observed an association of TAAGA haplotype with patients with worse lithium response even after correction for multiple testing (10 000 permutations) ($p$=0.003). The data regarding haplotype frequencies were shown in Table 5.

**Tab. 3.** Genotype and allele frequencies of the *GR* gene polymorphisms for bipolar patients with different lithium response (figures in parentheses indicate percentages).

| Polymorphism | | | ER | PR | NR | *p*-value |
|---|---|---|---|---|---|---|
| Rs41423247 | genotypes | GG | 2 (6.67) | 9 (15.52) | 5 (18.52) | 0.138 |
| | | CG | 8 (26.67) | 23 (39.66) | 13 (48.15) | |
| | | CC | 20 (66.66) | 26 (44.83) | 9 (33.33) | |
| | alleles | G | 12 (20.00) | 41 (35.34) | 23 (42.60) | 0.028* |
| | | C | 48 (80.00) | 75 (64.66) | 31 (57.40) | |
| Rs6195 | genotypes | CC | 29 (96.67) | 53 (91.38) | 23 (85.19) | 0.307 |
| | | CT | 1 (3.33) | 5 (8.62) | 4 (14.81) | |
| | | TT | 0 (0.00) | 0 (0.00) | 0 (0.00) | |
| | alleles | C | 59 (98.33) | 111 (95.70) | 50 (92.60) | 0.324 |
| | | T | 1 (1.70) | 5 (4.30) | 4 (7.40) | |
| Rs10052957 | genotypes | AA | 5 (16.67) | 8 (14.04) | 4 (14.81) | 0.784 |
| | | AG | 12 (40.00) | 21 (36.84) | 7 (25.93) | |
| | | GG | 13 (43.33) | 28 (49.12) | 16 (59.26) | |
| | alleles | A | 22 (36.67) | 37 (31.90) | 15 (27.78) | 0.599 |
| | | G | 38 (63.33) | 77 (68.10) | 39 (72.22) | |
| Rs6198 | genotypes | CC | 1 (3.33) | 2 (3.45) | 0 (0.00) | 0.627 |
| | | CT | 11 (36.67) | 17 (29.31) | 6 (22.22) | |
| | | TT | 18 (60.00) | 39 (67.24) | 21 (77.78) | |
| | alleles | C | 13 (21.67) | 21 (18.10) | 6 (11.10) | 0.318 |
| | | T | 47 (78.33) | 95 (81.90) | 48 (88.90) | |
| Rs6191 | genotypes | AA | 6 (20.70) | 13 (23.64) | 8 (29.63) | 0.314 |
| | | AC | 12 (41.38) | 32 (58.18) | 12 (44.44) | |
| | | CC | 11 (37.92) | 10 (18.18) | 7 (25.93) | |
| | alleles | A | 24 (41.40) | 58 (52.73) | 28 (51.85) | 0.348 |
| | | C | 34 (58.60) | 52 (42.27) | 26 (48.15) | |
| Rs258813 | genotypes | AA | 5 (16.67) | 8 (14.04) | 4 (14.81) | 0.784 |
| | | AT | 12 (40.00) | 21 (36.84) | 7 (25.93) | |
| | | TT | 13 (43.33) | 28 (49.12) | 16 (59.26) | |
| | alleles | A | 22 (36.67) | 37 (32.46) | 15 (27.78) | 0.599 |
| | | T | 38 (63.33) | 77 (67.54) | 39 (72.22) | |
| Rs6196 | genotypes | AA | 22 (73.33) | 46 (79.31) | 19 (70.37) | 0.617 |
| | | AG | 7 (23.33) | 8 (13.79) | 7 (25.93) | |
| | | GG | 1 (3.33) | 4 (6.90) | 1 (3.70) | |
| | alleles | A | 51 (85.00) | 100 (86.20) | 45 (83.33) | 0.884 |
| | | G | 9 (15.00) | 16 (13.80) | 9 (16.67) | |
| Rs33388 | genotypes | AA | 6 (20.00) | 14 (24.14) | 9 (36.00) | 0.150 |
| | | AT | 13 (43.33) | 34 (58.62) | 9 (36.00) | |
| | | TT | 11 (36.67) | 10 (17.24) | 7 (28.00) | |
| | alleles | A | 25 (41.67) | 62 (53.45) | 27 (54.00) | 0.283 |
| | | T | 35 (58.33) | 54 (46.55) | 23 (46.00) | |

* indicates statistical trend

## DISCUSSION

The results of our single marker analysis have shown a significant association of excellent response to lithium and C allele of rs41423247 polymorphism. Other SNPs have not revealed significant association with lithium response in our group of bipolar patients in single marker analysis.

The SNP rs41423247 (previously BclI) denominates C>G substitution located in intron 2, 647 bp from 3' side of exon 2. The minor G allele has been associated, among others, with lower cortisol levels after dexamethasone administration suggesting increased sensitivity to glucocorticoids and poor negative feedback regulation of HPA axis activity (Rosmond *et al.* 2000). This allele of BclI polymorphism was also associated with major depression (Krishnamurthy *et al.* 2008; van Rossum *et al.* 2006; Zobel *et al.* 2008) and poor response to antidepressant treatment (Brouwer *et al.* 2006). However, no *in vitro* data are available for this SNP, so the

**Tab. 4.** Genotype and allele frequencies of the *GR* gene polymorphisms for bipolar patients with excellent response vs. partial- and non-responders (figures in parentheses indicate percentages).

| Polymorphism | | | ER | PR+NR | *p*-value |
|---|---|---|---|---|---|
| Rs41423247 | genotypes | GG | 2 (6.67) | 14 (16.47) | 0.050 |
| | | CG | 8 (26.67) | 36 (42.35) | |
| | | CC | 20 (66.66) | 35 (41.18) | |
| | alleles | G | 12 (20.00) | 64 (37.00) | 0.016* |
| | | C | 48 (80.00) | 106 (63.00) | |
| Rs6195 | genotypes | CC | 29 (96.67) | 76 (89.41) | 0.225 |
| | | CT | 1 (3.33) | 9 (10.59) | |
| | | TT | 0 (0.00) | 0 (0.00) | |
| | alleles | C | 59 (98.33) | 161 (94.70) | 0.460 |
| | | T | 1 (1.70) | 9 (5.30) | |
| Rs10052957 | genotypes | AA | 5 (16.67) | 12 (14.29) | 0.695 |
| | | AG | 12 (40.00) | 28 (33.33) | |
| | | GG | 13 (43.33) | 44 (52.38) | |
| | alleles | A | 22 (36.67) | 52 (30.95) | 0.426 |
| | | G | 38 (63.33) | 116 (69.05) | |
| Rs6198 | genotypes | CC | 1 (3.33) | 2 (2.35) | 0.565 |
| | | CT | 11 (36.67) | 23 (27.06) | |
| | | TT | 18 (60.00) | 60 (70.59) | |
| | alleles | C | 13 (21.67) | 27 (15.88) | 0.325 |
| | | T | 47 (78.33) | 143 (84.12) | |
| Rs6191 | genotypes | AA | 6 (20.70) | 21 (25.61) | 0.185 |
| | | AC | 12 (41.38) | 44 (53.66) | |
| | | CC | 11 (37.92) | 17 (20.73) | |
| | alleles | A | 24 (41.40) | 86 (52.44) | 0.170 |
| | | C | 34 (58.60) | 78 (47.56) | |
| Rs258813 | genotypes | AA | 5 (16.67) | 12 (14.29) | 0.695 |
| | | AT | 12 (40.00) | 28 (33.33) | |
| | | TT | 13 (43.33) | 44 (52.38) | |
| | alleles | A | 22 (36.67) | 52 (30.95) | 0.426 |
| | | T | 38 (63.33) | 116 (69.05) | |
| Rs6196 | genotypes | AA | 22 (73.33) | 65 (76.47) | 0.711 |
| | | AG | 7 (23.33) | 15 (17.65) | |
| | | GG | 1 (3.33) | 5 (5.88) | |
| | alleles | A | 51 (85.00) | 145 (85.30) | 1.000 |
| | | G | 9 (15.00) | 25 (14.70) | |
| Rs33388 | genotypes | AA | 6 (20.00) | 23 (27.71) | 0.206 |
| | | AT | 13 (43.33) | 43 (51.81) | |
| | | TT | 11 (36.67) | 17 (20.48) | |
| | alleles | A | 25 (41.67) | 89 (53.61) | 0.132 |
| | | T | 35 (58.33) | 77 (46.39) | |

* indicates statistical significance

**Tab. 5.** Frequencies of haplotype blocks between the group of patients with excellent lithium responders versus patients with partial or no response to lithium.

| Hapotype | Haplotype frequency | PR+NR : ER frequency | $X^2$ | *p*-value |
|---|---|---|---|---|
| TCAGT | 0.496 | 0.465, 0.583 | 2.498 | 0.114 |
| TAAGA | 0.176 | 0.221, 0.050 | 8.937 | 0.002* |
| CAAAA | 0.171 | 0.155, 0.217 | 1.188 | 0.275 |
| TAGAA | 0.148 | 0.147, 0.150 | 0.003 | 0.956 |

* indicates statistical significance, after 10 000 permutations (correction for multiple testing): p = 0.003

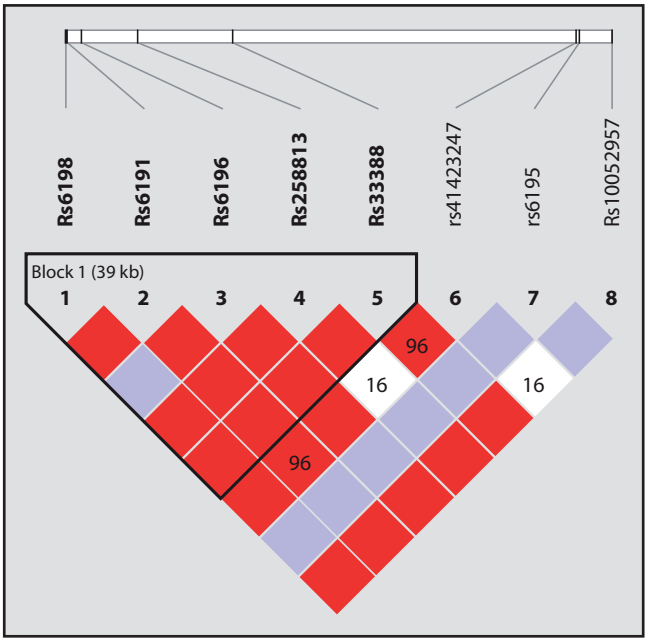


**Fig. 1.** Relative positions and LD estimates between glucocorticoid receptor gene polymorphisms in the analyzed population of bipolar patients. Colored squares correspond to D′ values with numerical estimates given within the squares (no value in the square means complete LD; haplotype block marked with black line).

exact mechanism through which BclI may alter glucocorticoids effects remains unclear. Its location does not involve any regulatory or splicing region of GR gene. It is, however, possible that this polymorphism is in linkage disequilibrium with other variations in GR gene that are important for gene function (Manenschijn *et al.* 2009). In our study, we have found that minor G allele was significantly more frequent among bipolar patients with partial and no response to lithium treatment. To date, there are no other studies analyzing the effect of GR polymorphism on lithium response, so it is not possible to verify the present results.

It is worth emphasizing that the BclI variant associated with poor response to antidepressants was also more frequent in the partial and non-responders to lithium in bipolar patients. The tendency observed in the present study is consistent with the previously published data, where BDNF Met allele or 5-HTT *short* variant, associated with poor response to antidepressants in depressive patients, were also more frequent in the group of bipolar patients with poor response to lithium (Rybakowski *et al.* 2005a; Rybakowski *et al.* 2005b).

We also performed linkage disequilibrium analysis and found that TAAGA haplotype is significantly more prevalent in the group of partial and non-responders to lithium. Therefore, we may assume that this haplotype is associated with worse lithium response. In the previous study we found that two polymorphisms of the haplotype block (rs6198 and rs6191) were associated with MDD susceptibility in our population (unpublished results). It is, therefore, plausible that this haplotype affects glucocorticoid receptor function that impairs lithium response.

The main limitation of our study is the relatively small number of patients for this type of genetic association research; therefore the power is not sufficient to exclude false positives and detect the true association. On the other hand, our patients were thoroughly characterized clinically and the duration of lithium administration (minimum 5 years) enabled precise assessment of the quality of lithium prophylactic effect.

Summarizing, the present study indicate the involvement of *GR* gene polymorphism in the response to lithium treatment in bipolar patients. However, the results should be interpreted cautiously taken into account limitations mentioned above and further data are necessary to confirm observed association.

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### *Conflict of interest*

*There are no potential conflicts of interest for any of the authors to the subject of the report.*

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