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Single nucleotide polymorphisms of *NR3C1* gene and recurrent depressive disorder in population of Poland

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Abstract Depressive disorder is a disease characterized by disturbances in the hypothalamo–pituitary–adrenal axis. Abnormalities include the increased level of glucocorticoids (GC) and changes in sensitivity to these hormones. The changes are related to glucocorticoid receptors gene (*NR3C1*) variants. The *NR3C1* gene is suggested to be a candidate gene affecting depressive disorder risk and management. The aim of this study was to investigate polymorphisms within the *NR3C1* gene and their role in the susceptibility to recurrent depressive disorder (rDD). 181 depressive patients and 149 healthy ethnically matched controls were included in the study. Single nucleotide polymorphisms were assessed using polymerase chain reaction/restriction fragment length polymorphism method. Statistical significance between rDD patients and controls was observed for the allele and genotype frequencies at three *loci*: *Bcl*I, N363S, and ER22/23EK. The presence of C allele, CC, and GC genotype of *Bcl*I polymorphism, G allele and GA genotype for N363S and ER22/23EK variants respectively were associated with increased rDD risk. Two haplotypes indicated higher susceptibility for rDD, while haplotype GAG played a protective role with OR_{dis} 0.29 [95 % confidence interval (CI) = 0.13–0.64]. Data generated from this study support the earlier results that genetic variants of the *NR3C1* gene are associated with rDD and suggest further consideration on the possible involvement of these variants in etiology of the disease.

Keywords Depressive disorder · Single nucleotide polymorphism · Glucocorticoid receptor

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Introduction

Depressive disorder is one of the most common and disabling psychiatric disorders. For a long time, a link between depression and the endocrine system has not only been suspected but confirmed, as well. The hypothalamic–pituitary–adrenal (HPA) axis is the most important element of the neuroendocrine involved in depression. There are ample evidence for the hyperactivity of HPA axis, altered feedback regulation and increased level of cortisol in unipolar depression [1]. In addition, there are also data informing about the decrease in number of glucocorticoid receptors (GRs) in depressive patients and their increase with antidepressant treatment [2, 3]. In contrast, GRs occurring in high number result in worse response to stress that often brings about depression symptoms [4].

Many peripheral and brain processes are affected by GC and GRs-related signals. These include control of inflammation [5], influence on cognition and synaptic plasticity, as well regulation of stress-response processes [6, 7]. Such results suggest a role of GC and GRs transduction pathways in mood disorders including depression, as the disease is characterized by the increased activity of immune/inflammatory pathways [8], disturbed cognition [9] and synaptic plasticity [10].

HPA-related disturbances, including higher level of cortisol, are not only typical for the patients suffering from depression. They are also characteristic for their first-degree relatives and might be a prodromal risk factor for the developing depression [11]. Moreover, HPA-axis dysfunction and HPA-related genes are associated with immune/inflammatory disorders [12]. There are also data informing that blood cells are resistant to GC [13] and it might possibly participate in the enhancement of inflammatory process.

The findings of the increased cortisol level in depression and the lack of suppression of this hormone with dexametasone prompted to a hypothesis that GRs might have altered sensitivity to GC. Disturbances of many brain related processes involving GRs and increased number of proinflammatory cytokines that influence GRs [14] may explain changes in expression of GRs and sensitivity to GC in depression. In addition, genes involved in the regulation of HPA axis [15] and GR function [16] are related to the risk of depression.

Different sensitivity of GRs and the receptors expression can also depend on the functional condition of the GR and *NR3C1* gene, encoding the molecule. In the healthy population, a group of polymorphisms within the gene for GRs was described. The three mainly known, important single nucleotide polymorphisms (SNPs) include *Bcl*I (rs414232 47, C>G), N363S (rs6195, A>G), and ER22/23EK (rs6189 and rs 6190; G>A). The *Bcl*I polymorphism was discovered as a restriction fragment and is determined by a cytosine to guanine substitution at position 647 in intron 2. The sensitivity to GC is in different way associated with that genetic variant. In addition, tissue-specific changes in sensitivity are observed [17–19]. The second variant N363S is located in codon 363 (exon 2). The variant is characterized by asparagine to serine change and is associated with the increased sensitivity to GC [20]. The ER22/ER23 polymorphisms are two linked nucleotide changes in codons 22 and 23. The first variant is silent, and glutamic acid is coded. The second variant results in arginine (R) to lysine(K) change. Presence of the ER22/23EK allele is related to the resistance to GC [21]. Additionally, C-reactive protein concentration [19] and changes in the balance between two isoforms of GR GRα ligand-dependent transcription factor and GRβ—having no transcriptional activity, was found to be related to the polymorphism [22, 23].

Considering the increasing need to replicate and extend genetic studies, we examined the *NR3C1* gene polymorphisms and haplotypes in Polish population. The aim of the present study has been to investigate whether there is an association between polymorphism in gene for GRs and the recurrent depressive disorders (rDD) development.

Materials and methods

Subjects

A group of 181 patients, treated for rDD (102 females; 56.4 %), were enrolled into the study. The mean age in that group was 42.6 ± 8.2 years (mean ± SD). The diagnosis was established, according to ICD-10 criteria (F33.0–F33.8) [24]. In all the qualified cases, medical history was obtained, using the standardized composite international diagnostic interview (CIDI) [24]. Additionally, the number of depressive episodes, duration of the disease and the age at onset were assessed in each patient. The control group consisted of 149 healthy subjects (83 females; 55.7 %) with family history negative for psychiatric disorders. The mean age in that group was 38.7 ± 6.7 years (mean ± SD). The control subjects included community volunteers, enrolled to the study following the criteria of the psychiatric CIDI interview [24]. Both patients and controls with other psychiatric diagnoses, concerning axis I and II disorders, were excluded from the study. All the patients and control subjects were native, unrelated inhabitants of the central Poland. An informed consent was obtained from all the participants of the study. The study protocol had earlier been approved by the Local Bioethics Committee No. RNN/626/09/KB.

No significant differences were found between analysed groups with respect to gender ($p > 0.05$). Hardy–Weinberg's distributions in the individual polymorphisms were as follows: *Bcl*I: polymorphism: for rDD ($\chi^2 - 7.08$; $p < 0.0001$); for controls ($\chi^2 - 2.47$; $p = 0.12$); N363S polymorphism: for rDD ($\chi^2 - 28.8$; $p < 0.0001$); for controls ($\chi^2 - 0.26$; $p = 0.61$); ER22/23EK polymorphism: for rDD ($\chi^2 - 0.41$; $p = 0.52$); for controls ($\chi^2 - 0.04$; $p = 0.84$).

Genotyping

DNA was extracted from whole blood according to the guanidine thiocyanate (GTC) method. Polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) for intronic *Bcl*I polymorphism was analyzed using 0.1 µg genomic DNA, 200 µM each dNTP, 5 × Go*Taq* buffer solution, 1 u Go*Taq* polymerase (Promega, Madison WI USA), 0.5 µM specific primers5′-GAGAAATTCACCCC TACCAAC-3′ and 5′-AGAGCCCTATTCTTCAAACTG-3′. After a 5 min denaturing step amplification was performed,

according to the following cycling profile: 94 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s (34 cycles). The final elongation step was 10 min at 72 °C. Amplification product 418 bp was digested with restriction enzyme *Bcl*I (New England BioLabs) at 50 °C for 10 h. The polymorphism was visualized by separating the digested amplification products on 2 % agarose gel. The amplified fragment was 418 bp long from which the restriction reaction yielded fragments of 267 and 151 bp for wild-type homozygous samples, fragments of 418, 267, and 151 bp for wild-type heterozygous samples, and a 418 bp fragment, which was detected in polymorphic (mutant) homozygotes.

Allele-specific PCR for N363S polymorphism was performed with the method designed by Majnik et al. [25]. Allele-specific PCR reaction yielded a control fragment of 357 bp in each tube and a specific fragment of 306 bp in those tubes where the wild type allele (coding asparagine) or the mutant allele (coding serine) corresponding to the applied specific primer was present. Primers sequences were 363 W: 5′-ATCCTTGGCACCTATTCCAAT-3′ corresponding to wild type and 363 M: 5′-ATCCTTGGCACC TATTCCAAC-3′ to mutant Ser allele. The polymorphism was visualized by separating the digested amplification products on 3 % agarose gel.

PCR-based RFLP for exonic ER22/23EK polymorphism was analyzed using 0.1 μg genomic DNA, 200 μM each dNTP, 5 × Go*Taq* buffer solution, 1 u Go*Taq* polymerase (Promega, Madison WI USA), 0.5 μM specific primers 5′-TGCATTCGGAGTTAACTAAAAG-3′ and 5′-ATCCC AGGTCATTTCCCATC-3′. After a 5 min denaturing step amplification was performed according to the following cycling profile: 94 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s (34 cycles). The final elongation step was 10 min at 72 °C. The amplified fragment was 448 bp long and was digested with *Mnl*I restriction enzyme (New England Biolabs) at 37 °C for 4 h. This yielded fragments of 149 and 163 bp (and smaller fragments of 50, 49, and 35 bp) in the presence of the wild-type allele and fragments of 163 and 184 bp (and smaller fragments of 50 and 49 bp) in the presence of the polymorphic allele. The RFLP products were separated and visualized on 3 % agarose gel.

In addition, 10 % of all samples were randomly selected and genotyped in duplicate, and the results were fully concordant.

Statistical analysis

The results are reported as percentages (%) or means with standard deviations (±SD). In order to examine the association between *NR3C1* gene polymorphisms and rDD, χ^2 test was used. Post hoc power analysis was performed with the use of non-central χ^2 distribution. The analysis of association was based on 95 % confidence interval (CI) for the disease odds ratio (OR_{dis}), calculated with use of logistic regression model including sex and age as covariates. Departures from the Hardy–Weinberg's equilibrium were determined by comparison of observed genotype prevalence rates with expected ones. In all the analyses, $p \leq 0.05$ was accepted as the level of statistical significance.

Results

Patients clinical characteristics and genotype distribution are shown in Table 1. In both groups, no significant relationships were found between the distributions of analysed genotypes and the demographic variables (age, sex). Relationships were not also observed between analysed genotypes and such clinical variables as the age at disease onset, disease duration and the number of episodes, except for duration of the rDD for N363S polymorphism (Kruskal–Walis' test; $p = 0.013$) (Table 1).

Data and statistical analyses regarding genotype (heterozygotes or homozygotes) distribution, allele frequency and OR_{dis} values (adjusted for age and sex) determined with respect to the *Bcl*I, N363S and ER22/23EK SNPs of the *NR3C1* gene are presented in Table 2. Significant differences were observed in genotype distribution and allele frequency between rDD-affected patients and controls (Table 2). In all the analysis post hoc power analysis showed high power of testing $1-\beta = 99$ %. When evaluating the *Bcl*I polymorphism, we demonstrated that presence GG homozygote and G allele significantly reduced the risk of rDD while presence of C allele and CC, GC genotypes were a positive factor of the disease risk. The obtained value of OR_{dis} for N363S polymorphism was significant and indicated protective role for A allele while G allele as risk factor for having rDD. Results, regarding the ER22/23EK were demonstrated that a GG heterozygote decreases the risk of rDD while GA increases the risk in question.

The haplotype analysis results are shown in Table 3. We found five haplotypes with significant differences between cases and controls. Two of them indicate higher susceptibility to rDD with OR_{dis}, ranged from 2.16 to 7.48, and one haplotype plays a protective role with OR_{dis} 0.29.

Discussion

The main aim of the present study has been to examine, whether genetic variants of *NR3C1* gene are associated with depression, especially with rDD in population of central Poland. The results of our study supported an association between three SNPs of the gene in question, indicating existence of the risky alleles. The haplotype analysis led to the detection of possible variants in which two of them represented vulnerability to rDD.

Table 1 Clinical characteristics of patients and genotype distribution

Genotype (*n*)	Age of onset (years)	Duration of the rDD in (years)	Number of episodes
*Bcl*I			
GG (42)	35 ± 5	7 ± 3	4 ± 1
GC (108)	34 ± 6	9 ± 5	4 ± 1
CC (31)	34 ± 6	9 ± 5	4 ± 1
N363S*			
AA (154)	34 ± 6	9 ± 4	4 ± 1
AG (19)	35 ± 6	9 ± 4	4 ± 1
GG (6)	31 ± 2	6 ± 2	3 ± 1
ER22/23EK			
GG (162)	34 ± 6	9 ± 4	4 ± 1
GC (18)	35 ± 5	9 ± 5	4 ± 1
CC (1)	36	7	4

rDD recurrent depressive disorders

* Significant differences between genotype distribution and duration of rDD for N363S polymorphism; *n* number of genotypes; Kruskal–Walis test; $p = 0.013$

The relationship between the *NR3G1* gene and depression was observed by other authors [26–28]. In Caucasian population van Rossum et al. [28] found that homozygous carriers of the B*cl*I polymorphism are more susceptible to depression and also found the significant differences in genotype frequency, what is noticeable in our data, as well. Krishnamurthy et al. [29] presented the similar results in a group of depressive premenopausal women. In addition, the presence of B*cl*I polymorphism was associated with lower decrease in Hamilton Rating Scale for depression (HDRS) [30]. An important role for the ER22/23EK carriers and the presence of A allele in patients were observed by van Rossum et al. [28], while we found increased risk of depression for the GA heterozygotes. Here, rather rare presence of AA genotype in Caucasian group should be mentioned. Moreover, post hoc power analysis in our study showed high power of testing. Brouwer et al. [30] results showed a little difference in genotype distribution in depressive Dutch patients (without the differentiation to unipolar depression).

Table 2 Genotypes and alleles frequencies of the *Bc1*l, N363S and ER22/23EK polymorphisms and risk of rDD

	rDD, *n* (%)	Controls, *n* (%)	OR (95 % CI)	*p*	OR* (95 % CI)	*p**
*Bcl*I polymorphism						
Genotypes $\chi2 = 24.3$ $p = 0.00001$						
GG	42 (23.2)	70 (47.0)	0.34 (0.21–0.55)	<0.00001	0.35 (0.22–0.58)	0.00002
GC	108 (59.7)	70 (47.0)	1.67 (1.08–2.59)	0.022	1.66 (1.05–2.62)	0.028
CC	31 (17.1)	9 (6.0)	3.21 (1.47–7.01)	0.003	3.08 (1.38–6.89)	0.006
Allele $\chi2 = 20.9$ $p < 0.00001$						
G	192 (53.0)	210 (70.5)	0.47 (0.34–0.65)	<0.00001	0.465 (0.33–0.65)	<0.00001
C	170 (47.0)	88 (29.5)	2.11 (1.53–2.92)	<0.00001	2.15 (1.53–3.00)	<0.00001
N363S polymorphism						
Genotype $\chi2 = 7.54$ $p = 0.023$						
AA	154 (85.1)	137 (92.0)	0.50 (0.24–1.03)	0.058	0.76 (0.37–1.55)	0.45
AG	19 (10.5)	12 (8.0)	1.34 (0.63–2.86)	0.45	1.32 (0.59–2.91)	0.46
GG	8 (4.4)	–				
Allele $\chi2 = 7.93$ $p = 0.005$						
A	327 (90.3)	286 (96.6)	0.39 (0.20–0.77)	0.0065	0.33 (0.16–0.67)	0.0020
G	35 (9.7)	12 (3.4)	2.56 (1.30–5.00)	0.0065	3.03 (1.49–6.25)	0.0020
ER22/23EK polymorphism						
Genotype $\chi2 = 6.36$ $p = 0.042$						
GG	162 (89.5)	144 (96.6)	0.30 (0.11–0.82)	0.018	0.28 (0.10–0.80)	0.017
GA	18 (9.9)	5 (3.4)	3.18 (1.15–8.82)	0.026	3.39 (1.18–9.75)	0.023
AA	1 (0.6)	–				
Allele $\chi2 = 6{,}64$ $p = 0.010$						
G	342 (94.5)	293 (98.3)	0.99 (0.70–1.40)	0.95	0.99 (0.71–1.37)	0.95
A	20 (5.5)	5 (1.7)	1.02 (0.71–1.43)	0.95	1.02 (0.73–1.41)	0.95

Odds ratio calculated with the use of logistic regression, *rDD* recurrent depressive disorders, *OR* odds ratio, *CI* confidence interval

* OR—odds ratio age and sex adjusted (with appropriate *p** value)

Table 3 Haplotypes frequencies of the *Bc1*l, N363S and ER22/23EK polymorphisms

	*Bc1*I	N363S	ER22/23	rDD, *n* (%)	Controls, *n* (%)	OR (95 % CI)	*p*
1	C	A	G	131 (72.4)	79 (53.0)	2.16 (1.34–3.48)	0.0003
2	C	A	A	12 (6.6)	1 (0.7)	7.48 (0.99–56.08)	0.049
3	C	G	G	26 (14.4)	4 (2.7)	7.23 (2.37–22.07)	0.00005
4	C	G	A	12 (6.6)	1 (0.7)	7.48 (0.99–56.08)	0.049
5	G	A	G	148 (81.8)	140 (94.0)	0.29 (0.13–0.64)	0.0022
6	G	A	A	2 (1.1)	5 (3.4)	0.42 (0.08–2.28)	0.31
7	G	G	G	8 (4.4)	11 (7.4)	0.69 (0.26–1.84)	0.45
8	G	G	A	4 (2.2)	4 (2.27)	1.15 (0.27–4.88)	0.84

rDD recurrent depressive disorders, *CI* confidence interval, % percentages

OR odds ratio adjusted for age and sex by method of logistic regression with 95 % confidence interval given in parentheses; *p* value of Wald statistics with χ^2 distribution

We found increased risk for the G allele for the N363S polymorphism, that is related to increased sensitivity to GC. Higher frequency of G allele was found by van Rossum et al. [28] in German patients suffering from depression. The relation between N362S variant was not observed by van West et al. [26], and by Szczepankiewicz et al. [27] in patients with depression, meeting DSM-IV criteria, while we used ICD-10. Possible source of differences could come from inclusion criteria.

We have also found that haplotype that consists of the major alleles is associated with depression risk. The relation between haplotype of *NR3C1* gene was observed earlier in depression by van Rossum et al. [28] and in patients with coronary heart disease suffering from depression [31].

Data obtained in our study corroborated previous results on involvement of *NR3C1* polymorphisms in depression risk in Caucasian population. Moreover, our results confirmed the need of replication and extension of previous genetic studies. As in earlier studies, we found the relation between rDD and SNPs that probably determined opposite effects of GC and GRs dependent signal transduction pathway. Such results confirm the heterogeneity of rDD etiology and open once again a discussion on the role of GC and GRs in depression management.

According to the results, indicating that N363S variant related to increased sensitivity to GC is a risk factor to depression, one may suggest the role of overactivation of GR and GC related mechanism in etiology of depression. There is an evidence that increased activation of GRs is followed by higher glutamate activity and distortion of neurogenesis—disturbances characteristic to the disease in question. In addition, the increased CRH expression in the brain regions related to depression, as an effect of positive feedback of GC and parallel occurrence of depressive symptoms, should be estimated [32].

ER22/23EK polymorphic allele affecting transcript level is related to the increased resistance to GRs but this allel increase the risk of depression, as well. In this case it appears as if the GC and GRs were important molecules for depression. The decrease in GRs expression and resistance to GC may result in increase of inflammatory activity, observed in depression [33]. ER22/23EK polymorphism seems to be modestly associated with a decreased risk for dementia, the latter is claimed to be connected with depression [34]. One may hypothesize and may suggest the important role of genotype dependent GR mechanism in depression, as in the inflammatory disease. Inflammatory nature of depressive disorder suggests that GC and GRs related signal plays a role in the disease susceptibility. According to Kuningas et al. [35] ER22/23EK variant is related to higher C-reactive protein level in patients with cardiovascular disease that often co-exists with depression. Van Vinsen et al. [36] demonstrates sensitivity of monocytes, that activity and number are increased in depression [37], in carriers of the mutant allel G of N363s variant. Moreover, polymorphic variant of *Bcl*I, associated with the reduced sensitivity of leucocytes to GC [17] is according to our data a risk factor for rDD. Allel G and GG homozygote was also found to be involved in higher inflammatory activity [12]. Above results support that inflammatory process in depression might be related to GS and GRs signal. Regarding the *Bcl*I variant, it should be considered that this polymorphism is intronic and has no functionality or is linked with functionally important variant. The *Bcl*I variant was not found to be associated with the depression risk in patients meeting DSM-IV criteria [27]. The polymorphism tissue specificity is also important. Nevertheless, in several inflammatory diseases, decreased sensitivity of blood cells to GC was observed [38, 39]. The role of other inflammatory—related genes cannot be excluded. Moreover, there are other genotype dependent and independent mechanisms, influencing the GR sensitivity. For example, changes in sensitivity might be determined by other gene variants [40]. There is also evidence for influence of interferon beta, cytokines and macrophage migration inhibitory factor on GR sensitivity and function [36, 41, 42].

Conclusion

The genetic variants, which are associated with GC sensitivity, may belong to factors leading to the depression development. The present study addressed and confirmed the importance of *NR3C1* gene polymorphism in rDD, especially in Caucasian population. The findings suggest that not only the increase but also the decrease in GRs related mechanism can participate in induction of depression. The opposite results emphasize the wide and multifactorial role of GC and GRs and suggest that the GRs-related transduction pathway and its effect should be taken into account, considering, considering various cells and different conditions.

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References

1. Tichomirowa MA, Keck ME, Schneider HJ, Paez-Pereda M, Renner U, Holsboer F, Stalla GK (2005) Endocrine disturbances in depression. J Endocrinol Invest 28:89–99
2. Pariante CM (2009) Risk factors for development of depression and psychosis. Glucocorticoid receptors and pituitary implications for treatment with antidepressant and glucocorticoids. Ann N Y Acad Sci 1179:144–152
3. Païzanis E, Renoir T, Lelievre V, Saurini F, Melfort M, Gabriel C, Barden N, Mocaër E, Hamon M, Lanfumey L (2010) Behavioural and neuroplastic effects of the new-generation antidepressant agomelatine compared to fluoxetine in glucocorticoid receptor-impaired mice. Int J Neuropsychopharmacol 13:759–774
4. van Zuiden M, Geuze E, Willemen HL, Vermetten E, Maas M, Heijnen CJ, Kavelaars A (2011) Pre-existing high glucocorticoid receptor number predicting development of posttraumatic stress symptoms after military deployment. Am J Psychiatry 168:89–96
5. Baschant U, Tuckermann J (2010) The role of the glucocorticoid receptor in inflammation and immunity. J Steroid Biochem Mol Biol 120:69–75
6. Datson NA, Speksnijder N, Mayer JL, Steenbergen PJ, Korobko O, Goeman J, de Kloet ER, Joëls M, Lucassen PJ (2012) The transcriptional response to chronic stress and glucocorticoid receptor blockade in the hippocampal dentate gyrus. Hippocampus 22:359–371
7. Gallagher P, Reid KS, Ferrier IN (2009) Neuropsychological functioning in health and mood disorder: modulation by glucocorticoids and their receptors. Psychoneuroendocrinology 34: S196–S207
8. Maes M, Galecki P, Chang YS, Berk M (2011) A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog Neuropsychopharmacol Biol Psychiatry 35:676–692
9. Gohier B, Ferracci L, Surguladze SA, Lawrence E, El Hage W, Kefi MZ, Allain P, Garre JB, Le Gall D (2009) Cognitive inhibition and working memory in unipolar depression. J Affect Disord 116:100–105
10. Holderbach R, Clark K, Moreau JL, Bischofberger J, Normann C (2007) Enhanced long-term synaptic depression in an animal model of depression. Biol Psychiatry 62:92–100
11. Modell S, Lauer CJ, Schreiber W, Huber J, Krieg JC, Holsboer F (1998) Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders. Neuropsychopharmacology 18:253–262
12. Kostik MM, Klyushina AA, Moskalenko MV, Scheplyagina LA, Larionova VI (2011) Glucocorticoid receptor gene polymorphism and juvenile idiopathic arthritis. Pediatr Rheumatol Online J 9:2
13. van Winsen LM, Muris DF, Polman CH, Dijkstra CD, van den Berg TK, Uitdehaag BM (2005) Sensitivity to glucocorticoids is decreased in relapsing remitting multiple sclerosis. J Clin Endocrinol Metab 90:734–740
14. Irusen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF, Adcock IM (2002) p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. J Allergy Clin Immunol 109:649–657
15. Liu Z, Zhu F, Wang G, Xiao Z, Tang J, Liu W, Wang H, Liu H, Wang X, Wu Y, Cao Z, Li W (2007) Association study of corticotropin-releasing hormone receptor1 gene polymorphisms and antidepressant response in major depressive disorders. Neurosci Lett 414:155–158
16. Brent D, Melhem N, Ferrell R, Emslie G, Wagner KD, Ryan N, Vitiello B, Birmaher B, Mayes T, Zelazny J, Onorato M, Devlin B, Clarke G, DeBar L, Keller M (2010) Association of FKBP5 polymorphisms with suicidal events in the treatment of resistant depression in adolescents (TORDIA) study. Am J Psychiatry 167:190–197
17. Panarelli M, Holloway CD, Fraser R, Connell JM, Ingram MC, Anderson NH, Kenyon CJ (1998) Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. J Clin Endocrinol Metab 83:1846–1852
18. van Rossum EF, Koper JW, van den Beld AW, Uitterlinden AG, Arp P, Ester W, Janssen JA, Brinkmann AO, de Jong FH, Grobbee DE, Pols HA, Lamberts SW (2003) Identification of the *Bcl*I polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. Clin Endocrinol (Oxf) 59:585–592
19. van Rossum EF, Feelders RA, van den Beld AW, Uitterlinden AG, Janssen JA, Ester W, Brinkmann AO, Grobbee DE, de Jong FH, Pols HA, Koper JW, Lamberts SW (2004) Association of the ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in elderly men. Am J Med 117:158–162
20. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, Grobbee DE, Brinkmann AO, De Jong FH, Lamberts SW (1998) A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab 83:144–151
21. Russcher H, van Rossum EF, de Jong FH, Brinkmann AO, Lamberts SW, Koper JW (2005) Increased expression of the glucocorticoid receptor-A translational isoform as a result of the ER22/23EK polymorphism. Mol Endocrinol 19:1687–1696
22. van den Akker EL, Russcher H, van Rossum EF, Brinkmann AO, de Jong FH, Hokken A, Pols HA, Koper JW, Lamberts SW (2006) Glucocorticoid receptor polymorphism affects transrepression but not transactivation. J Clin Endocrinol Metab 91:2800–2803
23. Anacker C, Zunszain PA, Carvalho LA, Pariante CM (2011) The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36:415–425
24. World Health Organization, 1992. The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Description and Diagnostic Guidelines
25. Majnik J, Patócs A, Balogh K, Tóth M, Rácz K (2004) A rapid and simple method for detection of Asn363Ser polymorphism of

the human glucocorticoid receptor gene. J Steroid Biochem Mol Biol 92:465–468
26. van West D, Van Den Eede F, Del-Favero J, Souery D, Norrback KF, Van Duijn C, Sluijs S, Adolfsson R, Mendlewicz J, Deboutte D, Van Broeckhoven C, Claes S (2006) Glucocorticoid receptor gene-based SNP analysis in patients with recurrent major depression. Neuropsychopharmacology 31:620–627
27. Szczepankiewicz A, Leszczyńska-Rodziewicz A, Pawlak J, Rajewska-Rager A, Dmitrzak-Weglarz M, Wilkosc M, Skibinska M, Hauser J (2011) Glucocorticoid receptor polymorphism is associated with major depression and predominance of depression in the course of bipolar disorder. J Affect Disord 134: 138–144
28. van Rossum EF, Binder EB, Majer M, Koper JW, Ising M, Modell S, Salyakina D, Lamberts SW, Holsboer F (2006) Polymorphisms of the glucocorticoid receptor gene and major depression. Biol Psychiatry 59:681–688
29. Krishnamurthy P, Romagni P, Torvik S, Gold PW, Charney DS, Detera-Wadleigh S, Cizza G, P.O.W.E.R. (Premenopausal, Osteoporosis Women, Alendronate, Depression) Study Group (2008) Glucocorticoid receptor gene polymorphisms in premenopausal women with major depression. Horm Metab Res 40:194–198
30. Brouwer JP, Appelhof BC, van Rossum EF, Koper JW, Fliers E, Huyser J, Schene AH, Tijssen JG, Van Dyck R, Lamberts SW, Wiersinga WM, Hoogendijk WJ (2006) Prediction of treatment response by HPA-axis and glucocorticoid receptor polymorphisms in major depression. Psychoneuroendocrinology 31:1154–1163
31. Otte C, Wüst S, Zhao S, Pawlikowska L, Kwok PY, Whooley MA (2009) Glucocorticoid receptor gene and depression in patients with coronary heart disease: the Heart and Soul Study-Curt Richter Award Winner. Psychoneuroendocrinology 34:1574–1581
32. Reul JM, Holsboer F (2002) Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. Curr Opin Pharmacol 2:23–33
33. Anisman H (2009) Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. J Psychiatry Neurosci 34:4–20
34. van Rossum EF, de Jong FJ, Koper JW, Uitterlinden AG, Prins ND, van Dijk EJ, Koudstaal PJ, Hofman A, de Jong FH, Lamberts SW, Breteler MM (2008) Glucocorticoid receptor variant and risk of dementia and white matter lesions. Neurobiol Aging 29:716–723
35. Kuningas M, Mooijaart SP, Slagboom PE, Westendorp RG, van Heemst D (2006) Genetic variants in the glucocorticoid receptor gene (NR3C1) and cardiovascular disease risk. The Leiden 85-plus Study. Biogerontology 7:231–238
36. van Winsen LL, Hooper-van Veen T, van Rossum EF, Polman CH, van den Berg TK, Koper JW, Uitdehaag BM (2005) The impact of glucocorticoid receptor gene polymorphisms on glucocorticoid sensitivity is outweighted in patients with multiple sclerosis. J Neuroimmunol 167:150–156
37. Maes M, Van der Planken M, Stevens WJ, Peeters D, DeClerck LS, Bridts CH, Schotte C, Cosyns P (1992) Leukocytosis, monocytosis and neutrophilia: hallmarks of severe depression. J Psychiatr Res 26:125–134
38. Kirkham BW, Corkill MM, Davison SC, Panayi GS (1991) Response to glucocorticoid treatment in rheumatoid arthritis: in vitro cell mediated immune assay predicts in vivo responses. J Rheumatol 18:821–825
39. Spahn JD, Landwehr LP, Nimmagadda S, Surs W, Leung DY, Szefler SJ (1996) Effects of glucocorticoids on lymphocyte activation in patients with steroid-sensitive and steroid-resistant asthma. J Allergy Clin Immunol 98:1073–1079
40. Zhao Y, Shen HY, Chen XY, Xiong RP, Li P, Liu P, Yang N, Zhou YG (2010) Genetic variations of heat shock protein 84 in mice mediate cellular glucocorticoid response. Cell Physiol Biochem 25:359–366
41. Aeberli D, Yang Y, Mansell A, Santos L, Leech M, Morand EF (2006) Endogenous macrophage migration inhibitory factor modulates glucocorticoid sensitivity in macrophages via effects on MAP kinase phosphatase-1 and p38 MAP kinase. FEBS Lett 580:974–981
42. Pace TW, Hu F, Miller AH (2007) Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun 21:9–19