Published in final edited form as:

Psychoneuroendocrinology. 2009 November ; 34(10): 1574–1581. doi:10.1016/j.psyneuen.2009.08.016.

Glucocorticoid receptor gene and depression in patients with coronary heart disease: The Heart and Soul Study—2009 Curt Richter Award Winner

Christian Otte^{a,*}, Stefan Wüst^b, Shoujun Zhao^c, Ludmila Pawlikowska^d, Pui-Yan Kwok^d, and Mary A. Whooley^{c,e,f}

^a Department of Psychiatry, University Medical Center Hamburg-Eppendorf, Germany

^b Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany

^c Veterans Affairs Medical Center, San Francisco, CA, USA

^d Institute for Human Genetics, University of California, San Francisco, CA, USA

^e Department of Medicine, University of California, San Francisco, CA, USA

^f Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA

Summary

Alterations of glucocorticoid receptor sensitivity have been associated with depression. Thus, variation in the glucocorticoid receptor gene that determines glucocorticoid sensitivity may influence risk for depression.

In a cross-sectional genetic association study of 526 white outpatients with chronic coronary heart disease, we examined whether haplotypes of the glucocorticoid receptor gene (NR3C1) are associated with depression. Participants were genotyped for four common glucocorticoid receptor gene polymorphisms (ER22/23EK, BclI C/G, N363S, and 9beta A/G) and haplotype analyses were conducted. Depression was assessed by an interview (Computerized Diagnostic Interview Schedule).

Of the 526 participants, 355 (67.5%) were non-carriers, 153 (29.1%) had one copy, and 17 (3.2%) had 2 copies of the haplotype 3 allele, which includes the minor allele of the 9beta A/G polymorphism and which has been associated with reduced glucocorticoid sensitivity. The prevalence of depression ranged from 24.4% in the non-carriers to 34.4% in heterozygotes to 52.9% in participants homozygous for the haplotype 3 allele ($p < 0.01$). In logistic regression analyses, carriers of one haplotype 3 allele had an odds ratio of 1.64 (95% CI 1.1–2.5, $p = 0.02$) for depression, while the odds ratio of homozygous haplotype 3 carriers was 3.52 (95% CI 1.3–9.4, $p = 0.01$). These associations persisted after adjusting for potentially confounding variables.

Keywords

Depression; Glucocorticoid receptor; Cortisol; Genetics; Stress

*Corresponding author at: University Hospital Hamburg-Eppendorf, Department of Psychiatry and Psychotherapy, Martinistrasse 52, 20246 Hamburg, Germany. Tel.: +49 40 42803 3223; fax: +49 40 42803 3461. otte@uke.uni-hamburg.de (C. Otte).

A common GR haplotype was associated with depression in a gene-dosage dependent manner and might be a vulnerability factor for depression.

Conflict of interest

All other authors declare that they have no conflicts of interest.

1. Introduction

In response to various stimuli, cortisol coordinates metabolic, endocrine, immune, and nervous system responses (McEwen, 1998; de Kloet et al., 2005). The effects of cortisol are mainly mediated by the glucocorticoid receptor (GR), which is expressed throughout the body including the brain (de Kloet et al., 2005).

Alterations in GR function leading to impaired negative feedback regulation have been implicated in the aetiology and pathogenesis of depression (Holsboer, 2000; Pariante and Miller, 2001; Nemeroff, 2004). Indeed, many patients with major depression exhibit decreased feedback inhibition of cortisol in the dexamethasone suppression test (Gold and Chrousos, 2002; de Kloet et al., 2005), a probe of GR function. Furthermore, suicide victims with major depression had reduced GR mRNA in the hippocampus and prefrontal cortex (Webster et al., 2002) and increased methylation of the GR gene promoter inhibiting GR expression (McGowan et al., 2009). Thus, depression appears to be associated with diminished GR function leading to decreased glucocorticoid sensitivity.

It is known that sensitivity to glucocorticoids varies considerably between individuals (Huizenga et al., 1998). Four common polymorphisms (ER22/23EK = rs6189, 6190, *BclI* C/G = rs41423247, N363S = rs6195, 9beta A/G = rs6198) (van Rossum and Lamberts, 2004; Wüst et al., 2004a,b; DeRijk and de Kloet, 2008) appear to modulate glucocorticoid sensitivity. Some of these polymorphisms have been associated with depression in medically healthy subjects but with inconsistent results (Binder et al., 2004; van Rossum et al., 2006; van West et al., 2006; Krishnamurthy et al., 2008; Zobel et al., 2008; Bet et al., 2009). To our knowledge, only one study simultaneously examined these polymorphisms with regard to depression. The Longitudinal Aging Study Amsterdam found a gene $\times$ environment interaction between the 9beta A/G polymorphism and childhood adversity on self-reported depressive symptoms in an elderly sample from the general population (Bet et al., 2009). However, no haplotype analyses were conducted. Interestingly, the 9beta A/G polymorphism has also been associated with the cortisol response to psychological stress (Kumsta et al., 2007).

Because of these previous findings, we hypothesized that GR haplotype 3, which includes the minor allele of the 9beta A/G polymorphism, would be associated with depression in a sample of 526 white patients with stable coronary disease who were enrolled in the Heart and Soul Study.

2. Methods

2.1. Participants

Details regarding our recruitment procedures have been published previously (Ruo et al., 2003; Otte et al., 2004, 2005, 2007; Whooley et al., 2008). We used administrative databases to identify outpatients with documented coronary disease at two Veterans Affairs Medical Centers (San Francisco VA Medical Center and the VA Palo Alto Health Care System, California), one University Medical Center (University of California, San Francisco), and nine public health clinics in the Community Health Network of San Francisco. Patients were eligible to participate if they had at least one of the following: a history of myocardial infarction, angiographic evidence of $\geq 50\%$ stenosis in one or more coronary vessels, prior evidence of exercise-induced ischemia by treadmill or nuclear testing, or a history of coronary revascularization. A total of 1024 participants enrolled and completed a daylong study appointment at the San Francisco VA Medical Center. To avoid population stratification (Schulze and Mahon, 2002; Hattersley and McCarthy, 2005), we chose to examine genotypes only in white patients, the largest group in the Heart and Soul study ($n = 595$). Our protocol

was approved by the appropriate institutional review boards. After complete description of the study to the subjects, written informed consent was obtained.

2.2. Depression

We measured the presence of current (past month) and past year major depression according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria. We used the modified Computerized National Institute of Mental Health Diagnostic Interview Schedule (CDIS-IV), a highly structured interview designed to yield psychiatric diagnoses (Robins et al., 1981). The DIS has been used extensively to study the epidemiology and treatment of depression (Wells et al., 1989). Throughout the manuscript we refer to “current or past year depression” as depression. Trained research assistants administered the interview during the daylong baseline study appointment. Participants found to have current depression were informed that they were suffering from depression, instructed to discuss these symptoms with their primary care provider, and provided a list of local resources available for further evaluation and treatment.

We also assessed depressive symptoms using the 9-item Patient Health Questionnaire (Spitzer et al., 1999), a self-report instrument that measures the frequency of depressive symptoms corresponding to the nine Diagnostic and Statistical Manual-IV criteria for depression. Participants indicated the frequency of experiencing each symptom during the prior 2 weeks, and the instrument was scored as: not at all (0), several days (1), more than half the days (2), or nearly every day (3).

2.3. Genotyping

2.3.1. DNA was extracted from white blood cells collected at the time of the interview—Polymorphism-spanning fragments were amplified by the polymerase chain reaction (PCR) and genotyped by template-directed dye-terminator incorporation assay with fluorescence polarization detection (FP-TDI) (Hsu and Kwok, 2003), using the AcycloPrime-FP II kit (Perkin-Elmer) per manufacturer’s instructions. Plates were read on the EnVision fluorescence polarization plate reader (Perkin-Elmer) and genotypes scored with software (Excel macro) provided by Perkin-Elmer. Standard PCR conditions: 5 µl reaction in 384-well plates, with 2.4 ng dried genomic DNA, 0.12–0.24 µM each primer, 0.1–0.2 U Platinum Taq (Invitrogen), 0.05 mmol/L dNTPs and 2.5–3.5 mmol/L MgCl₂. Cycling conditions: 95 °C for 2 min; 45 cycles of 92 °C for 10 s, 58 °C for 20 s, 68 °C for 30 s; followed by 68 °C for 10 min. All plates contained positive and negative controls. Genotyping was performed by investigators blinded to clinical status.

The four polymorphisms we examined (ER22/23EK = rs6190, *BclI* C/G = rs41423247, N363S = rs6195, 9beta A/G = rs 6198) are common variants of the glucocorticoid receptor gene (*NR3C1*) that have all been associated with the changes in glucocorticoid sensitivity (Wüst et al., 2004a,b; van Rossum et al., 2005). Fig. 1 schematically depicts the GR gene, the location of the four polymorphisms and their specific nucleotide variations.

Haplotypes as well as linkage disequilibrium among the four variants (with *D'* and *r*²) was estimated using the Haploview software. For each haplotype, 3 genotype combinations were distinguished as carrying 0, 1, or 2 copies of the haplotype allele.

2.4. 24-h urinary cortisol

Details regarding collecting 24-h urinary cortisol have been published previously (Otte et al., 2004). In brief, patients were instructed to collect all urine for 24 h between the end of their study appointment and the time when a researcher visited their house the next day. Research personnel arrived at patient homes exactly 24 h after their appointment to ensure accurately

timed specimens and to enhance compliance with the protocol. If subjects were unable to collect all urine for any reason or had urinary incontinence, their samples were deemed inadequate and no urinary cortisol data were recorded for these subjects.

Cortisol was analyzed using radioimmunoassay or (due to a change at the ARUP Lab) High Performance Liquid Chromatography/Tandem Mass Spectrometry.

2.5. Other variables

Age, sex, alcohol use, and smoking were determined by self-report. We assessed medical history using a self-report checklist that included 45 common medical diagnoses. Body mass index was calculated as weight in kilograms divided by the square of height in meters, and obesity was defined as body mass index ≥ 30 kg/m². Participants were instructed to bring their medication bottles to the study appointment, and study personnel recorded all current medications. Medications were categorized using Epocrates Rx (San Mateo, CA).

All participants underwent resting echocardiography using an Acuson Sequoia ultrasound System (Mountain View, CA). We obtained standard two-dimensional views and performed planimetry with a computerized digitization system to determine left ventricular ejection fraction (Lett et al., 2008).

Participants also completed an exercise treadmill test according to a standard Bruce protocol at the baseline examination. Those who were unable to continue the standard Bruce protocol were switched to slower settings and encouraged to exercise for as long as possible. Exercise capacity was calculated as the total number of metabolic equivalent tasks (METs) achieved.

2.6. Statistical analysis

The goal of this study was to examine the association of GR gene haplotypes with depression in patients with coronary heart disease. Differences in characteristics between carriers of 0, 1, or 2 copies of haplotype 1–5 were compared using univariate analysis of variance for continuous variables and Chi-square tests for dichotomous variables. Because in our sample there were no homozygous subjects for haplotypes 4 and 5, these haplotypes were analyzed as carriers (1 copy) and non-carriers (0 copies).

The proportion of participants with depression in carriers of 0, 1, or 2 haplotypes of interest was analyzed by Mantel-Haenszel Chi-square test. We also used logistic regression to examine the association between the glucocorticoid receptor haplotypes and depression.

To account for possible confounding, we computed odds ratios with 95% confidence interval in multivariate models: unadjusted (model 1), adjusted for age and sex (model 2), adjusted for model 2 and body mass index (model 3), and adjusted for model 3 and smoking (model 4).

For all statistical analyses, $P < 0.05$ was considered statistically significant.

Analyses were performed using Statistical Analysis Software (version 9, SAS Institute, Cary, NC).

3. Results

Among the 595 white participants, successful genotyping for all four polymorphisms was achieved in 526 participants from whom haplotypes were estimated (Fig. 1). Reasons for non-availability of DNA included failure of venipuncture, failure of DNA isolation, or failure of allelic discrimination. The genotype distribution for the four polymorphisms did not deviate significantly from Hardy-Weinberg-Equilibrium (all p -values > 0.4).

3.1. Haplotypes

The haplotype structure corresponded to those previously reported (Wüst et al., 2004b; van Rossum et al., 2006; Kumsta et al., 2007; van den Akker et al., 2008). The haplotype with the highest frequency (haplotype 1, 41.4%) consisted of the major alleles of the four SNPs. Haplotype 2 (34.8%) was characterized by the minor G allele of the *Bcl* polymorphism. Three of the four polymorphisms were found to be mutually exclusive; only the minor codon 23 A allele was always present in combination with the minor 9beta G allele (haplotype 4, 3.8%). However, the G allele of the exon 9beta SNP was also observed independently from ER22/23EK (haplotype 3, 17.9%). The minor A allele of the N363S SNP was present in haplotype 5 (2.1%).

3.2. Depression

Current (past month) or past year depression was present in 148/526 (28.1%) participants. We did not find any association between haplotype 1, 2, 4, or 5 and depression (all p -values > 0.21).

In contrast, haplotype 3 was associated with depression in a gene-dosage dependent manner (Fig. 2). Compared to 24.4% (86/355) of non-carriers, depression was present in 34.4% (53/154) of participants carrying one allele of haplotype 3 and 52.9% (9/17) of homozygous haplotype 3 carriers (Mantel-Haenszel Chi-square test: $p < 0.01$).

In logistic regression analyses, carriers of one haplotype 3 allele had an odds ratio of 1.64 (95% CI 1.1–2.5, $p = 0.02$) for depression, while the odds ratio of homozygous haplotype 3 carriers was 3.52 (95% CI 1.3–9.4, $p = 0.01$). These associations persisted after adjusting for age, sex, body mass index, and smoking (Table 1).

With regard to self-reported depressive symptoms we also found increasing PHQ scores with numbers of haplotype 3 alleles on a descriptive but non-significant level (effect size: Cohen's $d = 0.34$, homozygous carriers compared to non-carriers, Table 2). Self-reported anxiety and perceived stress did not differ between haplotype 3 carriers and non-carriers (Table 2).

3.3. 24-h urinary cortisol

Neither haplotype 3 nor other haplotypes (data not shown) were associated with 24-h urinary cortisol values although homozygous haplotype 3 carriers had numerically higher 24-h urinary cortisol levels compared to non-homozygotes (43 ± 30 µg/day vs. 36 ± 23 µg/day, $p = 0.37$, effect size: Cohen's $d = 0.26$).

4. Discussion

We found that a common glucocorticoid receptor (GR) haplotype, which includes the minor allele of the 9beta A/G polymorphism, is associated with depression. Furthermore, we found a gene-dosage effect: carriers of one allele of GR haplotype 3 had a more than 60% increased risk for depression, while homozygous GR haplotype 3 carriers even had a more than 3-fold increased risk for depression compared to non-carriers. These associations persisted after controlling for potentially confounding variables.

Our findings suggest that GR haplotype 3 might be a vulnerability factor for depression in patients with coronary heart disease. Furthermore, our results are consistent with the hypothesis that differences in cortisol sensitivity are involved in the aetiology and pathogenesis of depression as postulated by the glucocorticoid receptor hypothesis of depression (Holsboer, 2000). Our results are also compatible with earlier findings that have demonstrated (1) decreased feedback inhibition of cortisol in depressed patients in the dexamethasone

suppression test, a probe of GR function (Nelson and Davis, 1997), (2) reduced GR mRNA in the hippocampus and prefrontal cortex (Webster et al., 2002) or increased methylation of the GR gene promoter inhibiting GR expression (McGowan et al., 2009) in suicide victims with depression, and (3) increased depressive-like behaviour and elevated corticosterone in transgenic mice with forebrain specific disruption of GR function (Boyle et al., 2005; Ridder et al., 2005). All of these studies support the idea of diminished GR function leading to decreased glucocorticoid sensitivity in depression.

Thus, the 9beta A/G polymorphism, which was associated with depression in our study, is a plausible candidate gene because it leads to relative glucocorticoid resistance, i.e. reduced glucocorticoid sensitivity. This has been shown by functional studies that revealed a stabilizing effect of this polymorphism on GRbeta mRNA in vitro, possibly leading to enhanced expression of GRbeta protein (DeRijk et al., 2001). GRbeta is one of several GR protein isoforms and is generated through an alternative splicing pathway. In contrast to the functionally active and most abundant isoform GRalpha (Hagendorf et al., 2005), GRbeta is unable to bind ligand and is transcriptionally inactive (Yudt et al., 2003). We suggest that presence of this haplotype leads to a reduced quantity of functional GRalpha protein and to a relatively higher level of GRbeta protein, potentially increasing the GRbeta/GRalpha ratio. The expected effects on systemic level would be a relative insufficient glucocorticoid signaling, which could then lead to depression. Indeed, it has been shown that depressed patients had reduced expression of GRalpha mRNA in peripheral blood cells (Matsubara et al., 2006).

Earlier studies have examined the association between GR polymorphisms and depression but did not include the 9beta A/G polymorphism (Binder et al., 2004; van Rossum et al., 2006; van West et al., 2006; Krishnamurthy et al., 2008; Zobel et al., 2008). To our knowledge, only one study has simultaneously examined 9beta A/G along with the other functionally relevant polymorphisms with regard to depression. That study found a gene $\times$ environment interaction between the 9beta A/G polymorphism and childhood adversity on self-reported depressive symptoms in an elderly sample from the general population (Bet et al., 2009). Our results are consistent with these earlier findings. Furthermore, we extend these findings by demonstrating a direct effect of GR haplotype 3 on major depression in a clinical population of patients with coronary heart disease. Our findings also fit very well with results showing that 9beta A/G carriers exhibited an increased cortisol response to stress as well as increased ACTH values in the dexamethasone suppression test (Kumsta et al., 2007).

Two earlier studies found an association of the ER 22/23 polymorphism and depression (van Rossum et al., 2006; van West et al., 2006) but the 9beta A/G was not measured in these studies and no haplotype analyses were conducted.

However, the authors of the former study measured another (NR3C1-1) polymorphism in the GR promoter that is in complete linkage with the 9beta A/G (Kumsta et al., 2009). This means that the minor allele of the NR3C1-1 polymorphisms is always inherited with the minor allele of the 9beta A/G. Therefore it is possible to extrapolate their results to the 9beta A/G polymorphism. Their findings were equivocal with the minor NR3C-1 allele (and the minor G allele of 9beta A/G) being overrepresented in the controls in one but not another sample (van West et al., 2006).

In our study, haplotype 3 was more strongly associated with major depression than with self-reported depressive symptoms. Furthermore, haplotype 3 was not associated with self-reported anxiety and perceived stress. This might suggest that haplotype 3 is a rather specific risk factor for the development of major depression, at least in the presence of chronic medical illness. In contrast, our results do not support a more general role of the GR gene in self-reported perceived stress and anxiety.

Several limitations of our study should be considered. Only 17 participants in our sample were homozygous for haplotype 3, limiting our power to detect small to medium effect sizes. This might be one reason why we did not find an association of haplotype 3 with 24-h urinary cortisol secretion. Furthermore, previous studies found an effect of 9beta on basal cortisol secretion (van Schoor et al., 2007) and on cortisol responses to stress (Kumsta et al., 2007). However, in both studies this effect was only present in women but not men. The vast majority of our participants were men and we were not able to systematically examine genotype $\times$ sex. Therefore, our results are not necessarily applicable to women.

Some studies suggest that the association between variants of the GR gene might be stronger in patients with recurrent depression (van Rossum et al., 2006). We did not determine number of previous depressive episodes in our participants. Therefore, we cannot distinguish between patients who have recurrent depression vs. those with a first depressive episode.

In summary, we found that a common haplotype of the GR gene (including the minor allele of the 9beta A/G polymorphism) is associated with depression. Thus, variation in the glucocorticoid receptor gene may be involved in the pathogenesis of depression.

Acknowledgments

Role of funding source

This work was supported by grants from the Department of Veterans Affairs (VA) Epidemiology Merit Review Program, the VA Health Services Research and Development Service, the Robert Wood Johnson Foundation (Generalist Physician Faculty Scholars Program), the American Federation for Aging Research (Paul Beeson Faculty Scholars in Aging Research Program), and the Ischemia Research and Education Foundation. Dr. Otte was supported by a Young Investigator Award from the National Alliance for Research in Schizophrenia and Depression (NARSAD). None of these funding sources had any role in the collection of data, interpretation of results, or preparation of this manuscript.

References

- Bet PM, Penninx BWJH, Bochdanovits Z, Uitterlinden AG, Beekman ATF, Schoor NMv, Deeg DJH, Hoogendijk WJG. Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression: New evidence for a gene-environment interaction. *Am J Med Genet B: Neuropsychiatr Genet* 2009;150B:660–669. [PubMed: 19051288]
- Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Kunzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Kohnlein O, Dabitz H, Bruckl T, Muller N, Pfister H, Lieb R, Mueller JC, Lohmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet* 2004;36:1319. [PubMed: 15565110]
- Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y, Muglia LJ. Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proc Natl Acad Sci* 2005;102:473–478. [PubMed: 15623560]
- de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005;6:463–475. [PubMed: 15891777]
- DeRijk RH, de Kloet ER. Corticosteroid receptor polymorphisms: determinants of vulnerability and resilience. *Eur J Pharmacol* 2008;583:303. [PubMed: 18321483]
- DeRijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, de Kloet ER, Emery P, Sternberg EM, Detera-Wadleigh SD. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *J Rheumatol* 2001;28:2383–2388. [PubMed: 11708406]

- Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry* 2002;7:254–275. [PubMed: 11920153]
- Hagendorf A, Koper JW, de Jong FH, Brinkmann AO, Lamberts SWJ, Feelders RA. Expression of the human glucocorticoid receptor splice variants {alpha}, {beta}, and P in peripheral blood mononuclear leukocytes in healthy controls and in patients with hyper- and hypocortisolism. *J Clin Endocrinol Metab* 2005;90:6237–6243. [PubMed: 16118334]
- Hattersley A, McCarthy M. What makes a good genetic association study? *Lancet* 2005;366:1315–1323. [PubMed: 16214603]
- Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacol* 2000;23:477–501.
- Hsu T, Kwok P. Homogeneous primer extension assay with fluorescence polarization detection. *Methods Mol Biol* 2003;212:177–187. [PubMed: 12491910]
- Huizenga NATM, Koper JW, de Lange P, Pols HAP, Stolk RP, Grobbee DE, de Jong FH, Lamberts SWJ. Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo–pituitary–adrenal axis to a low dose of dexamethasone in elderly individuals. *J Clin Endocrinol Metab* 1998;83:47–54. [PubMed: 9435415]
- Krishnamurthy P, Romagni P, Torvik S, Gold PW, Charney DS, Detera-Wadleigh S, Cizza G, POWERSG. Glucocorticoid receptor gene polymorphisms in premenopausal women with major depression. *Horm Metab Res* 2008;40:194–198. [PubMed: 18246526]
- Kumsta D, Moser Streit F, Koper JW, Meyer J, Wüst S. Characterization of a glucocorticoid receptor gene (GR, NR3C1) promoter polymorphism reveals functionality and extends a haplotype with putative clinical relevance. *Am J Med Genet B: Neuropsychiatr Genet* 2009;150B:476–482. [PubMed: 18663733]
- Kumsta R, Entringer S, Koper JW, van Rossum EFC, Hellhammer DH, Wüst S. Sex specific associations between common glucocorticoid receptor gene variants and hypothalamus–pituitary–adrenal axis responses to psychosocial stress. *Biol Psychiatry* 2007;62:863. [PubMed: 17716631]
- Lett H, Ali S, Whooley M. Depression and cardiac function in patients with stable coronary heart disease: findings from the Heart and Soul Study. *Psychosom Med*. 2008
- Matsubara T, Funato H, Kobayashi A, Nobumoto M, Watanabe Y. Reduced glucocorticoid receptor alpha expression in mood disorder patients and first-degree relatives. *Biol Psychiatry* 2006;59:689–695. [PubMed: 16458268]
- McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* 1998;338:171–179. [PubMed: 9428819]
- McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 2009;12:342. [PubMed: 19234457]
- Nelson JC, Davis JM. DST studies in psychotic depression: a meta-analysis. *Am J Psychiatry* 1997;154:1497–1503. [PubMed: 9356556]
- Nemeroff CB. Neurobiological consequences of childhood trauma. *J Clin Psychiatry* 2004;65 (Suppl 1): 18–28. [PubMed: 14728093]
- Otte C, Marmar CR, Pipkin SS, Moos R, Browner WS, Whooley MA. Depression and 24-hour urinary cortisol in medical outpatients with coronary heart disease: The Heart and Soul Study. *Biol Psychiatry* 2004;56:241–247. [PubMed: 15312811]
- Otte C, McCaffery J, Ali S, Whooley MA. Association of a serotonin transporter polymorphism (5-HTTLPR) with depression, perceived stress, and norepinephrine in patients with coronary disease: The Heart and Soul Study. *Am J Psychiatry* 2007;164:1379–1384. [PubMed: 17728423]
- Otte C, Neylan TC, Pipkin SS, Browner WS, Whooley MA. Depressive symptoms and 24-hour urinary norepinephrine excretion levels in patients with coronary disease: findings from the Heart and Soul Study. *Am J Psychiatry* 2005;162:2139–2145. [PubMed: 16263855]
- Pariente CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry* 2001;49:391–404. [PubMed: 11274650]
- Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, Zink M, Hortnagl H, Flor H, Henn FA, Schutz G, Gass P. Mice with genetically altered glucocorticoid receptor expression show altered

- sensitivity for stress-induced depressive reactions. *J Neurosci* 2005;25:6243–6250. [PubMed: 15987954]
- Robins LN, Helzer JE, Croughan J, Ratcliff KS. National institute of mental health diagnostic interview schedule. its history, characteristics, and validity. *Arch Gen Psychiatry* 1981;38:381–389. [PubMed: 6260053]
- Ruo B, Rumsfeld JS, Hlatky MA, Liu H, Browner WS, Whooley MA. Depressive symptoms and health-related quality of life: the Heart and Soul Study. *JAMA* 2003;290:215–221. [PubMed: 12851276]
- Schulze T, Mahon FM. Genetic association mapping at the crossroads: which test and why? Overview and practical guidelines. *Am J Med Genet B: Neuropsychiatr Genet* 2002;114:1–11.
- Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders Patient Health Questionnaire. *JAMA* 1999;282:1737–1744. [PubMed: 10568646]
- van den Akker ELT, Koper JW, van Rossum EFC, Dekker MJH, Russcher H, de Jong FH, Uitterlinden AG, Hofman A, Pols HA, Witteman JCM, Lamberts SWJ. Glucocorticoid receptor gene and risk of cardiovascular disease. *Arch Intern Med* 2008;168:33–39. [PubMed: 18195193]
- van Rossum EFC, Lamberts SWJ. Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. *Recent Prog Horm Res* 2004;59:333–357. [PubMed: 14749509]
- van Rossum EFC, Russcher H, Lamberts SWJ. Genetic polymorphisms and multifactorial diseases: facts and fallacies revealed by the glucocorticoid receptor gene. *Trends Endocrinol Metab* 2005;16:445. [PubMed: 16275120]
- van Rossum E, Binder M, Majer J, Koper M, Ising S, Modell D, Salyakina S, Lamberts F, Holsboer. Polymorphisms of the glucocorticoid receptor gene and major depression. *Biol Psychiatry* 2006;59:681–688. [PubMed: 16580345]
- van Schoor NM, Dennison E, Lips P, Uitterlinden A, Cooper C. Serum fasting cortisol in relation to bone, and the role of genetic variations in the glucocorticoid receptor. *Clin Endocrinol* 2007;67:871–878.
- van West D, Eede JVD, Del-Favero J, Souery D, Norrback K, Duijn CV, Sluijs S, Adolfsson R, Mendlewicz J, Deboutte D, Broeckhoven CV, Claes S. Glucocorticoid receptor gene-based SNP analysis in patients with recurrent major depression. *Neuropsychopharmacology* 2006;31:620–627. [PubMed: 16192984]
- Webster MJ, Knable MB, O'Grady J, Orthmann J, Weickert CS. Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. *Mol Psychiatry* 2002;7:985–994. 924. [PubMed: 12399952]
- Wells KB, Hays RD, Burnam MA, Rogers W, Greenfield S, Ware JE Jr. Detection of depressive disorder for patients receiving prepaid or fee-for-service care. Results from the Medical Outcomes Study. *JAMA* 1989;262:3298–3302. [PubMed: 2585674]
- Whooley MA, de Jonge P, Vittinghoff E, Otte C, Moos R, Carney RM, Ali S, Dowray S, Na B, Feldman MD, Schiller NB, Browner WS. Depressive symptoms, health behaviors, and risk of cardiovascular events in patients with coronary heart disease. *JAMA* 2008;300:2379–2388. [PubMed: 19033588]
- Wüst S, Federenko IS, Rossum EFC, Koper JW, Kumsta R, Entringer S, Hellhammer DH. A psychobiological perspective on genetic determinants of hypothalamus–pituitary–adrenal axis activity. *Ann NY Acad Sci* 2004a;1032:52–62.
- Wüst S, van Rossum EFC, Federenko IS, Koper JW, Kumsta R, Hellhammer DH. Common polymorphisms in the glucocorticoid receptor gene are associated with adrenocortical responses to psychosocial stress. *J Clin Endocrinol Metab* 2004b;89:565–573.
- Yudt MR, Jewell CM, Bienstock RJ, Cidlowski JA. Molecular origins for the dominant negative function of human glucocorticoid receptor beta. *Mol Cell Biol* 2003;23:4319–4330. [PubMed: 12773573]
- Zobel A, Jessen F, vonWiddern O, Schuhmacher A, Höfels S, Metten M, Rietschel M, Scheef L, Block W, Becker T, Schild H, Maier W, Schwab S. Unipolar depression and hippocampal volume: impact of DNA sequence variants of the glucocorticoid receptor gene. *Am J Med Genet B: Neuropsychiatr Genet* 2008;147B:836–843. [PubMed: 18286599]

Figure 1.

Schematic overview of the glucocorticoid receptor (GR) gene polymorphisms and haplotypes. Haplotypes are numbered in order of decreasing frequency.

Figure 2. Proportion of participants with past month or past year depression according to number of GR gene haplotype 3 copies (Mantel-Haenszel Chi-square test: $p < 0.01$).

Table 1

Characteristics of 526 white Heart and Soul Study participants according to numbers of haplotype 3 of the glucocorticoid receptor gene.

	0 copies, <i>n</i> = 355	1 copy, <i>n</i> = 154	2 copies, <i>n</i> = 17	<i>p</i> -Value
Demographic				
Age	67.6 ± 10.9	67.9 ± 11.0	65.2 ± 12.7	0.62
Male	306 (86)	126 (82)	14 (82)	0.43
Medical history				
MI	199 (56)	86 (56)	8 (47)	0.75
Stroke	49 (14)	18 (12)	1 (6)	0.55
Diabetes	72 (20)	34 (22)	3 (18)	0.86
Medication use				
Antidepressant	76 (21)	31 (20)	4 (24)	0.92
ACE inhibitor	174 (49)	91 (59)	8 (47)	0.10
Beta blocker	199 (56)	96 (62)	9 (53)	0.39
Statins	234 (66)	102 (66)	9 (53)	0.54
Cardiac disease severity				
METS	7.6 ± 3.4	7.3 ± 3.8	6.8 ± 3.2	0.56
LVEF	0.62 ± 0.09	0.62 ± 0.10	0.61 ± 0.11	0.97
Other characteristics				
BMI	28.6 ± 4.8	28.1 ± 5.0	30.8 ± 11.1	0.10
Regular alcohol use	114 (32)	60 (39)	7 (41)	0.27
Smoking	48 (14)	34 (22)	5 (29)	0.02
Perceived stress	5.02 ± 3.29	5.39 ± 3.27	5.41 ± 3.59	0.50
PHQ score	4.7 ± 5.2	5.2 ± 5.1	6.5 ± 5.4	0.28
HADS anxiety score	5.34 ± 3.88	5.21 ± 3.91	5.34 ± 3.72	0.94

Percentages are shown in parentheses. METS: metabolic equivalents; LVEF: left ventricular ejection fraction.

Table 2

Unadjusted and adjusted logistic regression analyses with numbers of haplotype 3 copies as predictor variable and past month or past year depression as outcome variable.

Model	# copies of haplotype 3 allele	Odds ratio	95% CI	p-Value
1	0	Reference	–	–
	1	1.64	(1.1–2.5)	0.02
	2	3.52	(1.3–9.4)	0.01
2	0	Reference	–	–
	1	1.72	(1.1–2.7)	0.02
	2	3.76	(1.3–11.2)	0.02
3	0	Reference	–	–
	1	1.75	(1.1–2.7)	0.01
	2	3.73	(1.2–11.3)	0.02
4	0	Reference	–	–
	1	1.67	(1.1–2.6)	0.02
	2	3.44	(1.1–10.6)	0.03

Model 1: Unadjusted.

Model 2: Age- and sex-adjusted.

Model 3: Body mass index (BMI), age- and sex-adjusted.

Model 4: Smoking status, BMI, age- and sex-adjusted.