Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression: New evidence for a gene-environment interaction

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ABSTRACT

**Background:** The hypothalamic-pituitary-adrenal (HPA)-axis regulates the response to stressful events and is expected to be involved in the pathogenesis of depression. The glucocorticoid receptor (GR) regulates the activity of the HPA-axis. Both GR gene polymorphisms and childhood adversity are known to be associated with increased risk for depression.

**Methods:** In the Longitudinal Aging Study Amsterdam, a large population based sample of older men and women, 906 subjects were genotyped. An association study was performed to determine the relationship between GR gene polymorphisms, childhood adversity, HPA-axis markers and depressive symptoms.

**Results:** A gene-environment interaction between the GR gene polymorphisms 22/23EK and 9beta and childhood adversity resulted in an increased risk of clinically relevant depressive symptoms. Without childhood adversity no increased risk was present. The 22/23EK variant was also associated with a lower Free Cortisol Index in the presence of childhood adversity. Persons, that are heterozygous for the BclI variant, in contrast with wildtype and BclI-homozygotes, had lower serum levels of cortisol binding globulin and had no increased risk of recurrent depressive symptoms in the presence of childhood adversity.

**Conclusions:** We found a gene-environment (G x E) interaction between common variants of the GR gene and childhood adversity, demonstrating a vulnerable phenotype for developing clinically relevant depressive symptoms at old age. This G x E interaction also influenced HPA-axis markers providing support for the involvement of the HPA-axis in both stress regulation and the pathogenesis of depression.
INTRODUCTION

Major depressive disorder (MDD) is one of the most common psychiatric diseases with a one year prevalence of 5% and a lifetime prevalence up to 17%.[1,2] The World Health Organization (WHO) Global Burden of Disease Survey estimates that by the year 2020, MDD will be second only to ischemic heart disease in the amount of disability experienced by sufferers.[3] The recurrence of MDD is high, ranging from 41% in five years to 67% in ten years follow-up.[4,5] Subthreshold depression, also referred to as minor depression, subsyndromal depression, or significant depressive symptoms, is gaining interest of researchers and clinician for several reasons. The prevalence of subthreshold depression is high (5-15% in primary care) and it is an important risk factor for developing MDD. Subthreshold depression is often chronic or recurrent and the functional status of patients is impaired for many years.[6-9]

Several lines of evidence indicate that disturbances of the hypothalamus-pituitary-adrenal (HPA) axis are involved in the pathogenesis of depression[10] and the risk of recurrence.[11] The glucocorticoid receptor, (GR) (NR3C1) and mineralocorticoid receptor (MR) play an important role in HPA-axis and stress-regulation. The GR mediates many of the effects of cortisol on target tissues via direct binding to hormone-responsive elements in the DNA and via interactions with other transcription factors resulting in a modulation of gene transcription.[12,13] A cell’s response to cortisol is predominantly determined by both the cortisol level it is exposed to and by its GR-sensitivity.[12] Evidence from recent studies suggests that polymorphisms of the GR gene are a major factor in the large interindividual variability of HPA activity, serum levels of HPA-axis markers and GC sensitivity of target tissues in the population.[13] Four GR gene polymorphisms have been reported to influence GR-sensitivity which in its turn may be associated with affective disorders. The 22/23EK polymorphism (rs6189 and rs6190)[14] has been associated with a decreased GR-sensitivity.[15,16] The N363S A/G SNP (rs6195) has been associated with increased cortisol suppression, as well as with an increased insulin response after dexamethasone administration.[14,17] BclI[18] has recently been characterized as a C/G SNP (rs41423247) was also associated with increased cortisol supression after oral dexamethasone.[19,20] A fourth A/G SNP is in exon 9beta (rs6198) and could contribute to a decreased GR-sensitivity by increasing GRbeta-mRNA stability.[21]

Epidemiological data has provided information on the direct associations of these functional GR gene polymorphisms and affective disorders. Van West et al.[22] reported both allel and genotype associations between recurrent MDD and 22/23EK in a Swedish sample (n=315). This finding however could not be replicated in a Belgian sample (n=353).[22] Van Rossum et al.[23] recently reported associations between 22/23EK, N363S and the Bcll-homozygous subjects and recurrent unipolar depression.
Although genetic factors account for 40-70% of the risk for developing depression, many environmental factors, like stressful life events in childhood, have been implicated. Stressful life events in childhood, such as physical and sexual abuse, parental loss and other traumatic experiences, are often referred to as childhood adversity. Childhood adversity leads to a vulnerable phenotype for developing depression in later life. It is argued that the pathogenesis of multifactorial diseases like depression is the result of the interplay of common variants in many genes in combination with environmental factors. The landmark study of Caspi et al. showed such a gene-environment interaction in depression, involving the serotonin transporter gene promoter polymorphism and stressful life events. Knowing that both the GR genotype and childhood adversity are able to influence the HPA-axis and depression, it is interesting to investigate a possible gene-environment (G x E) interaction.

We performed an association study on 221 persons with clinically relevant depressive symptoms, of which 64 persons had recurrent depressive symptoms, and 685 non-depressed controls with four GR gene polymorphisms: 22/23EK, N363S, BclI and 9beta., We also explored a potential gene-environment (G x E) interaction of the GR genotype and childhood adversity in determining depressive symptoms and serum levels of HPA-axis markers (i.e., cortisol and cortisol binding globulin).

**METHODS AND MATERIALS**

**Sampling and study design**

Data for this study were collected in the Longitudinal Aging Study Amsterdam (LASA), which is an ongoing cohort study on predictors and consequences of changes in autonomy and well-being in the ageing population in the Netherlands. The sampling and data collection procedures and non-response have been described in detail elsewhere. In short, a random sample of men and women aged 55-85, stratified by sex, urbanization and expected five-year mortality, was drawn from the population registries of eleven municipalities in three regions in the Netherlands. For the present study, persons who participated in the assessments in 1995/96 and in 1992/93, and were born in or before 1930 (aged 65 years and older as of January 1, 1996) were selected (n=1509). In 1995/96 morning blood samples were obtained in 1352 persons. DNA collection was insufficient in one of our three study regions leading to a loss of 402 samples. 28 DNA-samples were excluded due to mistakes in the laboratory handling, gender inconsistencies and non-Caucasian origin, resulting into GR genotype assessments in 922 persons. For 16 persons, depressive symptoms data was incomplete resulting in 906 subjects for depressive symptoms analysis. Informed consent was obtained from all respondents and the study was approved by the Ethical Review Board of the VU University Medical Center.
Depression
Depressive symptoms were established at the assessments in 1995/96 and the assessments three years earlier in 1992/93. The Center for Epidemiological Studies-Depression Scale (CES-D) was used for all respondents. The time frame for assessing depressive symptoms with the CES-D scale was the past week. A CES-D score of 16 or above marks a period of clinically relevant depressive symptoms. At this cut-off, the criterion validity of the CES-D for MDD (1 month recency) was excellent (sensitivity 100%, specificity 88%). All persons scoring at or above the cut-off CES-D score of 16 at one of these two assessments were classified as having clinically relevant depressive symptoms, DS. All persons scoring two times at or above 16 were classified as having recurrent depressive symptoms, RDS, which may be related to chronicity of depression. All controls had scores below 16 at both assessments. In this article both DS and RDS are referred to as depression.

Co-morbidity
Co-morbidity was determined by asking the patient specifically for conditions, like diabetes, heart disease and stroke. The Mini-Mental-State-Examination (MMSE) was used to measure cognitive impairment.

Genotyping
Genomic DNA was extracted from samples of peripheral venous blood according to standard procedures. Genotypes were determined using the Taqman allelic discrimination assay. The Assay-by-Design service (http://www.appliedbiosystems.com) was used to set up a Taqman allelic discrimination assay for 22/23EK, N363S, 9beta and BclI. Primer and probe sequences are available on request. The PCR reaction mixture included 5 ng of genomic DNA in a 2 ul volume and the following reagents: FAM and VIC probes (200 nM), primers (0.9 uM), 2x Taqman PCR master mix (ABgene). PCR cycling reactions were performed in 384 wells PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Nieuwerkerk a/d Ijssel, the Netherlands) and consisted of initial denaturation for 15 minutes at 95°C, and 40 cycles with denaturation of 15 seconds at 95°C and annealing and extension for 60 seconds at 60°C. Results were analysed by the ABI Taqman 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc.). To confirm the accuracy of genotyping results, 80 randomly selected samples were genotyped again with the same method as a control. No discrepancies were detected. All laboratory procedures were carried out blind to case-control status.
Childhood adversity

Information on childhood adversity (CHA) was obtained in 1990/1991 by asking whether persons had experienced personal life events during their youth (before age of 18) that had a lasting effect on the rest of their lives. The answers included war experiences, impaired physical health, death or separation of parents and sexual abuse. Depression symptoms were measured three and six years after the childhood adversity measurement, in 1992/1993 and 1995/1996 respectively. This diminishes possible bias of recollection of childhood adversity by current depressed mood.

HPA-axis markers

Blood sampling for cortisol and cortisol binding globulin (CBG) serum levels was performed in 1995/1996. Respondents were invited to a health care center near their homes, where blood samples were collected in the morning between 8.30 and 10.00 AM. Participants were allowed to take tea and dry toast for breakfast, but no sugar or dairy products. Participants had a resting time of at least 15 minutes before sampling. The blood samples were centrifuged and serum was stored at 70 °C for approximately 7 years until processing with commercially available kits. Serum cortisol was determined by immunoassay (ACS, Centauer, Bayer Diagnostics, Mijdrecht, The Netherlands). CBG levels were determined using a radio immunoassay from Medgenix Diagnostics (Fleunes, Belgium). Results were expressed as nmol/L (cortisol) and mg/L (CBG). The free cortisol index (FCI) was calculated as total cortisol/CBG ratio to represent the biologically active fraction of cortisol.34,35

Statistical analysis

All genotyping results were tested for Hardy-Weinberg equilibrium (HWE). Calculation of pairwise linkage disequilibrium (LD) between the SNPs was carried out and we used R-square to describe the magnitude of LD. We have performed an initial Chi-square analysis on allele frequencies to test the association between alleles and our composite depression measures in a simple additive genetic model. All these analysis were performed with Haploview (version 3.2).36 Multivariate analysis associating genetic polymorphisms and CHA with depression and cortisol levels were performed with SPSS (version 15.0). Logistic regression analysis, adjusted for sex, age, diabetes, heart disease, stroke and MMSE were conducted to calculate Odds Ratio (OR) for the presence of depression by genotype. Analysis of covariance (ANCOVA) adjusted for sex, age and Body Mass Index (BMI), calculated adjusted mean scores of FCI, cortisol and CBG levels across genotype. Due to the limited number of homozygote variants, heterozygote and homozygote carriers were analyzed as one group for the 22/23EK, N363S and 9beta variants for our SPSS analysis. This can be referred to as a dominant genetic model. Because of the relatively high frequency heterozygote and homozygote carriers of the Bcll variants, Bcll variants were analyzed separately comparing both groups separately.
with the group of homozygote non-carriers. With this analysis we did not choose a specific genetic model.

Gene environment interaction testing was performed by creating a binary variant*CHA interaction term for each GR gene variant and a separate Bcl1 heterozygote*CHA interaction term for comparing the Bcl1-heterozygotes with both Bcl1 homozygotes. Interaction analysis was adjusted for the direct effects of CHA and the GR gene variant involved. We have used the statistical package R (version 2.6.2) to perform permutation analysis with 1000 tests on the interaction adjusted for the direct effects of CHA and 22/23EK. Gene environment correlation between GR gene variants and CHA was tested with a two-tailed Pearson correlation test. We took multiple testing in to consideration by investigating only a limited number of associations (i.e. 4 SNPs with DS, RDS, FCI and SNP*CHA-interactions) and using a p-value cutoff of 0.05 for all test.

RESULTS

Demographics
Of the 906 persons in the study sample, 221 (24%) reported DS at one or both of the study assessments. Of these 64 persons were classified as recurrent cases, RDS. The main characteristics in 1995/1996 are provided according to depression group (see Table 1). The persons with depression were older, were more often female, had more childhood adversity, lower cortisol, lower FCI-values, more cardiovascular disease and lower MMSE-scores than non-depressed controls.

Allele frequencies and Linkage Disequilibrium (LD)
Minor allel frequencies were 4% for the 22/23EK polymorphism, 3% for N363S, 35% for BclI and 17% for 9beta. The minor allel frequencies in our control sample were similar to other non-depressed population samples. All SNPs were found to be in Hardy Weinberg Equilibrium in our population (p > 0.10). Inter-marker LD scores expressed in R-square were all below 0.2 indicating no LD between the polymorphisms. However, all alleles with a 22/23EK polymorphism also contained a 9beta polymorphism (see Table 2).

Genetic polymorphisms analysis
Single marker analysis showed a significant association between the BclI variant and RDS. Minor allele frequencies of the BclI variant in RDS persons and non-depressed controls were 26.2% and 35% respectively (Chi-square=3.9, p=0.05). No other associations were found of DS and RDS with the other GR gene variants.
### Table 1  Demographic characteristics (mean ± SD) of persons with recurrent depressive symptoms, depressive symptoms and non-depressed controls.

<table>
<thead>
<tr>
<th></th>
<th>Depressive Symptoms</th>
<th>Non-depressed Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recurrent&lt;sup&gt;a&lt;/sup&gt;</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>n=64</td>
<td>p-value&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>78 (6.9)</td>
<td>.005</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>22%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Childhood adversity</td>
<td>48%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CES-D 1992/93 (points)</td>
<td>24.4 (7.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CES-D 1995/96 (points)</td>
<td>23.1 (6.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI (in kg/m²)</td>
<td>27.4 (4.4)</td>
<td>.31</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>456 (176)</td>
<td>.064</td>
</tr>
<tr>
<td>CBG (mg/l)</td>
<td>44.2 (8.8)</td>
<td>.017</td>
</tr>
<tr>
<td>Free Cortisol Index</td>
<td>10.5 (4.2)</td>
<td>.001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>6%</td>
<td>.70</td>
</tr>
<tr>
<td>Heart disease (%)</td>
<td>44%</td>
<td>.001</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>8%</td>
<td>.88</td>
</tr>
<tr>
<td>MMSE score (points)</td>
<td>26.3 (3.0)</td>
<td>.008</td>
</tr>
</tbody>
</table>

<sup>a</sup> subgroup from all persons with depressive symptoms  
<sup>b</sup> differences tested with Chi Square and Students T-test compared with non-depressed controls

### Table 2  Estimated haplotype frequencies of patients with recurrent depressive symptoms, depressive symptoms and non-depressed controls.

<table>
<thead>
<tr>
<th></th>
<th>Depressive Symptoms</th>
<th>Non-depressed Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recurrent&lt;sup&gt;a&lt;/sup&gt;</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>freq.</td>
<td>Chi-2</td>
</tr>
<tr>
<td>22/23EK N363S BclI 9beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G A C A</td>
<td>0.509</td>
<td>1.9</td>
</tr>
<tr>
<td>G A G A</td>
<td>0.264</td>
<td>3.9</td>
</tr>
<tr>
<td>G A C G</td>
<td>0.170</td>
<td>1.4</td>
</tr>
<tr>
<td>A A C G</td>
<td>0.017</td>
<td>1.4</td>
</tr>
<tr>
<td>G G C A</td>
<td>0.040</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> subgroup from all patients with depressive symptoms  
<sup>b</sup> differences tested with Chi Square compared with non-depressed controls

We observed five major haplotypes that accounted for more than 99% of all possible marker combinations in our sample (see Table 2). The prevalence of the different haplotypes were similar to the sample of Stevens et al.<sup>20</sup> Alleles without 22/23EK and Bcll were more prevalent in RDS persons than non-depressed controls, being 72.0% and 61.2% respectively (Chi-square=5.8, p=0.02).

The GR genotype-depression associations were further analysed with a multivariate model using logistic regression adjusted for sex and age. The three persons homozygous for the 22/23EK variant showed a twelve fold increased risk of having DS compared to persons
without the 22/23EK variant (OR: 13.4, 95%CI: 1.15-156, p=0.04). There was no association between one 22/23EK variant and DS. No other significant associations were found between RDS and DS and the other GR gene variants.

### Genetic polymorphisms and childhood adversity: depression

Our population was divided into persons with (n= 244, 27%) and without CHA (n= 657, 73%). Within these two groups the relationship between the four GR gene variants and phenotypes, RDS and DS, was examined. Due to the limited number of homozygote variants, heterozygote and homozygote carriers were analysed as one group for the 22/23EK, N363S and 9beta variants. Because of the relatively high frequency heterozygote and homozygote carriers of the Bcll variants were analyzed separately. We adjusted multivariate models for sex, age, diabetes, heart disease, stroke and MMSE.

Interaction analysis revealed significant associations for 22/23EK*CHA-interaction (OR=6.8, 95%CI: 1.4 – 32, p=0.02) and 9beta*CHA-interaction (OR=2.2, 95%CI: 1.04 – 4.8, p=0.04) with CHA in determining the risk of DS (see Table 3). Permutation analysis with 1000 tests on the 22/23EK*CHA-interaction adjusted for the direct effects of 22/23EK-variant and CHA confirmed this interaction with an empirical p-value of 0.016.

Because all 22/23EK variant alleles contain the 9beta variant, we have performed an interaction analysis in the subgroup of persons with the 9beta variant and without 22/23EK. No interaction was shown (OR=1.5, 95%CI: 0.7 – 3.5, p=0.31).

CHA increased the risk of DS twofold (OR=2.1, 95%CI: 1.5 – 3.0, p<0.001). Carrying a variant allele in combination with CHA suggests a tendency to an increased risk of DS for 22/23EK as well as 9beta, carrying a variant allele without the presence of CHA suggests a tendency to a decreased risk of DS (see Figure 1). The analysis of RDS data provided the same pattern of odds ratios, though not statistically significant, for 22/23EK and 9beta.

Significantly increased risks of RDS were observed in persons with CHA carrying no or two Bcll variants compared to persons without CHA and no variant alleles (see Figure 2). In the CHA-group carriers of one Bcll variant showed a decreased risk of RDS (OR=0.25, 95%CI: 0.09 - 0.72, p=0.01) compared to persons without the variant. Carriers of two Bcll variants showed no change in risk of RDS compared to persons without the variant (OR=0.95, 95%CI: 0.20 – 4.56, p=0.94). In persons without CHA, carrying one or two variants did not change the risk of RDS. In Bcll heterozygote * CHA interaction analysis the effect of carrying one Bcll variant in combination with CHA was significantly different from carrying no or two variants (OR=0.24, 95%CI: 0.07-0.85, p=0.03).
Table 3  Adjusted Odds Ratios (%95CI) of having depressive symptoms (DS) in persons carrying GR variants in the total sample, in the group with childhood adversity (CHA) and in the group without CHA.

<table>
<thead>
<tr>
<th>GR variant</th>
<th>Total (n=906)</th>
<th>with CHA (n=241)</th>
<th>without CHA (n=649)</th>
<th>CHA x GR variant interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%DS</td>
<td>OR (95%CI)</td>
<td>n</td>
</tr>
<tr>
<td>22/23EK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>841</td>
<td>24.9</td>
<td>ref</td>
<td>224</td>
</tr>
<tr>
<td>1,2</td>
<td>62</td>
<td>17.7</td>
<td>0.69 (0.35-1.39)</td>
<td>15</td>
</tr>
<tr>
<td>N363S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>839</td>
<td>24.0</td>
<td>ref</td>
<td>220</td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td>28.1</td>
<td>1.43 (0.79–2.58)</td>
<td>20</td>
</tr>
<tr>
<td>BclI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>386</td>
<td>25.1</td>
<td>ref</td>
<td>106</td>
</tr>
<tr>
<td>1</td>
<td>401</td>
<td>24.2</td>
<td>1.00 (0.71-1.40)</td>
<td>111</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>22.7</td>
<td>0.82 (0.49-1.39)</td>
<td>22</td>
</tr>
<tr>
<td>9 beta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>631</td>
<td>25.2</td>
<td>ref</td>
<td>168</td>
</tr>
<tr>
<td>1,2</td>
<td>274</td>
<td>22.6</td>
<td>0.88 (0.62-1.25)</td>
<td>73</td>
</tr>
</tbody>
</table>

a adjusted for sex, age, diabetes, heart disease, stroke and MMSE
b interaction testing based on the interaction term for CHA*GR variant, adjusted for the direct effects of CHA and the GR variant
c the nature of this interaction is shown in Figure 1
Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression

Figure 1 Adjusted Odds Ratios of Depressive Symptoms in persons with 0, 1 and 2 22/23EK and 9beta variants, without and with childhood adversity (CHA)
Each dot shows the mean value and each line shows the 95% confidence interval within a category
a adjusted for sex, age, diabetes, heart disease, stroke and MMSE
b number of GR variants in a subject
c CHA*GR variant interaction, adjusted for the direct effects of CHA and the GR variant

Figure 2 Adjusted Odds ratios of Recurrent Depressive Symptoms in persons with 0, 1 and 2 BclI variants, without and with childhood adversity (CHA)
Each dot shows the mean value and each line shows the 95% confidence interval within a category
a adjusted for sex, age, diabetes, heart disease, stroke and MMSE
b number of GR variants in a subject
c BclI heterozygote * CHA interaction analysis comparing one BclI variant with no or two BclI variants, adjusted for the direct effects of CHA and the BclI variant
d upper value of 95%CI = 25.1
Genetic polymorphisms and childhood adversity: HPA-axis

The GR gene variants were not associated with significant changes in FCI. GR gene variant \* CHA interaction analysis showed a significant association between the 22/23EK variant and CHA (see Table 4).

### Table 4
Free Cortisol Index (FCI) in persons carrying GR variants and CHA interaction analysis.

<table>
<thead>
<tr>
<th>GR variant</th>
<th>nr</th>
<th>Mean FCI(\text{a} ) (nmol/mg)</th>
<th>p-value</th>
<th>p-value interaction(\text{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/23EK</td>
<td>0</td>
<td>12.2 (11.9-12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1, 2</td>
<td>12.4 (11.3-13.4)</td>
<td>0.72</td>
<td>0.04(\text{c} )</td>
</tr>
<tr>
<td>N363S</td>
<td>0</td>
<td>12.3 (12.0-12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.6 (10.5-12.6)</td>
<td>0.22</td>
<td>0.72</td>
</tr>
<tr>
<td>BclI</td>
<td>0</td>
<td>12.1 (11.6-12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12.2 (11.8-12.6)</td>
<td>0.63</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.5 (11.7-13.3)</td>
<td>0.32</td>
<td>0.77</td>
</tr>
<tr>
<td>9beta</td>
<td>0</td>
<td>12.3 (12.0-12.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1, 2</td>
<td>11.9 (11.4-12.4)</td>
<td>0.20</td>
<td>0.66</td>
</tr>
</tbody>
</table>

\(\text{a}\) values are adjusted for sex, age and BMI (95% confidence interval)

\(\text{b}\) interaction testing based on the interaction term for CHA x GR variant, adjusted for the direct effects of CHA and the GR variant

\(\text{c}\) the nature of this interaction is shown in Figure 3

In persons with CHA the 22/23EK variant was associated with a trend to lower cortisol and higher CBG levels suggesting a tendency to lower FCI (10.1 versus 11.9 nmol co/mg CBG, \(p=0.10\)). In persons without CHA, there was no significant trend (FCI=13.1 versus 12.3 nmol co/mg CBG, \(p=0.21\)) (see Figure 3).

Persons carrying one BclI variant had lower CBG serum levels compared with persons carrying no or two variants, 40.7 mg/l versus 42.2 and 42.0 mg/l (adjusted for sex, age and BMI, \(F=7.6, p=0.006\)). This difference in CBG levels was present in persons with CHA (\(F=3.4, p=0.07\)) as well as persons without CHA (\(F=4.7, p=0.03\)) though statistical significance was not sustained in the smaller CHA-group. No other associations were found between cortisol, CBG and the other GR gene variants.

Genetic polymorphisms and childhood adversity: Gene environment correlation

No significant correlations were found between the GR gene variants and CHA in our cohort.
Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression

Figure 3 Adjusted Free Cortisol Index (FCI in nmol cortisol/mg CBG) in persons without and with 22/23EK mutations, without and with childhood adversity (CHA). Each box shows the median value and the interquartile range and each line shows the 100% range within a category
a adjusted for BMI, age and sex
b number of GR variants in a subject
c 22/23EK*CHA interaction, adjusted for the direct effects of 22/23EK and CHA

**DISCUSSION**

Our results provide evidence for a gene-environment interaction between the 22/23EK and the 9beta variant in the GR gene and the effect of childhood adversity on depression. In persons carrying the 22/23EK variant we also found an effect of childhood adversity on cortisol and CBG levels resulting in a significant decrease in Free Cortisol Index. Finally we showed that the persons, heterozygous for the Bcll variant, have lower CBG serum levels and are less vulnerable for depression in conjunction with childhood adversity, than Bcll-homozygotes and wildtype.

There is ample evidence that persons who have experienced stressful childhood adversity have an increased risk of depression in later life and at old age. We found the influence of childhood adversity on the prevalence of depression to be distinctly larger in persons...
carrying the 22/23EK or the 9beta variant. Because all 22/23EK variants contain the 9beta variant, we have explored the contribution of these two variants in this G x E interaction. Interaction analysis in the subgroup of persons with the 9beta variant and without 22/23EK, showed no statistical significance, indicating a prominent role for the 22/23EK variant. We did not find any evidence of increased risk of depression in persons with GR gene variant without childhood adversity. In fact, all odds ratios were below 1 except for the N363S variant. Our findings on childhood adversity increases the need to take this variable into account in GR genotype depression association studies and might help to explain inconsistent results as seen in association studies of the 22/23EK variant and recurrent MDD in a large Belgian and Swedish sample.22

In this sample depression was associated with lower Free Cortisol Index. This was due to lower cortisol levels and higher CBG-levels (see Table 1). Both hyper- and hypoactivity of the HPA-axis are associated with depressive symptoms and depression in late-life.43-46 Hypoactivity of the HPA-axis is associated with the clinical diagnosis of atypical depression47,48 and conditions that are associated with aging, like physical frailty, fatigue and pain.43,49,50 Although FCI was not influenced by GR gene variants alone, a significant G x E interaction was found between 22/23EK and childhood adversity on FCI. The combination of the 22/23EK and childhood adversity was associated with lower FCI, due to lower cortisol and higher CBG-levels. This is very much in agreement with an increased risk of depression in this sample. In the absence of childhood adversity there is no demonstrable difference in both FCI and risk of depression for the 22/23EK variant.

Caspi et al.29 already showed epidemiological data emphasizing the importance of the serotonin transporter polymorphism as a modulator of stress reactions and depression vulnerability. Recently Kim-Cohen et al. showed the influence of the MAOA genotype on the vulnerability to environmental stress (i.e. maltreatment) in predicting children’s mental health.51 We now show a G x E interaction between the GR gene and childhood adversity resulting in a vulnerable phenotype for developing depression at old age. The same G x E interaction accounts for lower FCI thus combining epidemiological measures with biological data in the same population in a consistent manner. This further strengthens the role of the GR receptor in stress reactivity and the role of the polymorphisms in the functionality of the HPA-axis.

In order to understand the increased vulnerability due to this G x E interaction of the 22/23EK variant and childhood adversity in our older sample further research must be performed on the relationship between CHA, the 22/23EK variant and glucocorticoid sensitivity. The 22/23EK variant has been associated with increased glucocorticoid sensitivity in an older
Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression

Increased glucocorticoid sensitivity is thought to normalize cortisol levels after stress more quickly and is believed therefore to diminish vulnerability for stress related diseases like depression. One could speculate that in later life increased glucocorticoid sensitivity could have a further cortisol lowering effect resulting in more hypocortisolemia and depression.

Recently van Rossum et al. showed increased risks for depression as well as recurrent unipolar depression in BclI homozygous persons. In our sample SNP and haplotype analysis revealed no increased risk of recurrent depressive symptoms in persons with the BclI variant after correction for the confounders sex and age. However, we found persons heterozygous for the BclI variant to be less vulnerable for recurrent depressive symptoms in conjunction with childhood adversity and to have lower CBG serum levels. Wust et al. showed that the heterozygous BclI variant was associated with a higher cortisol response to the Trier Social Stress Test and to ACTH application whereas homozygous BclI variants were associated with a lower cortisol response. Tremblay et al. showed an association a marked increase in weight gain over a period of 12 years in young females who where heterozygous for the BclI variant. These HPA-axis and metabolic differences between one or two BclI variants support the existence of a separate BclI heterozygous phenotype. Other examples of heterozygous phenotypes are known from mice models. Cyclo-oxygenase-2 and glucose-transporter-4 heterozygosity lead to obesity and diabetes respectively, whereas homozygosity and wildtype do not. Though the mechanism of a differential effect of BclI heterozygosity on the HPA-axis is not known, an explanation might be found in the highly tissue-specific effects, with both increased as well as decreased GR-sensitivity. This proposed BclI heterozygous phenotype, which is linked to distinct differences in recurrent depressive symptoms, HPA-axis markers, GR-sensitivity and obesity is an intriguing finding in the current views on the shared biology of obesity and depression.

A limitation of this study is the limited number of persons with recurrent depressive symptoms. Larger numbers of extreme phenotypes in this type of population studies are needed to increase the power to detect less distinct associations. Another limitation is that no life time indicator is available in this study. Life time depression acquired with a structured interview could have made the associations we found even stronger. The cortisol blood samples were taken in the morning hours, so there is a variable lag time between the early morning cortisol rise and the sample time. This limitation decreases the chance of detecting small differences between subgroups.

Childhood adversity data was collected retrospectively without using a structured interview, which may have resulted in underreporting. On the other hand, one could also argue, that by asking respondents about important life events in a rather open way at older
age, selectively brings out the most relevant events with large impacts on person's lives and the highest relevance in this type of research. No depression measurements were performed at the time of childhood adversity measurements, so it is not feasible to address the issue of recollection bias by current depressed mode. However it seems highly unlikely that this will interfere with the GxE-interaction we have found. For the recollection bias to influence this GxE-interaction, depressed subjects with the GR gene variant should recall more childhood adversity compared to depressed subjects without the GR gene variant. The absence of gene environment correlations between the GR gene variants and childhood adversity and our cohort study design provides a solid basis for finding true gene environment interactions.

The LASA study provides excellent longitudinal data of a large well defined cohort of the older Dutch population. The large group of non-depressed controls is screened with the CESD-scale on two separate occasions, three years apart, for depressive symptoms as well as MMSE-scale and cardiovascular and neurological co-morbidity. The prevalence of CIDI PTSD diagnosis was only 0.8 % and 1.0 % respectively in follow-up measurements 3 and 6 years after our depression and cortisol measurements. This makes our cohort an excellent reference group for epidemiologic depression research in the older population.

In summary, we found a gene-environment interaction between common variants of the GR gene and childhood adversity, demonstrating a vulnerable phenotype for developing clinically relevant depressive symptoms at old age. This G x E interaction also influenced HPA-axis markers providing support for the involvement of the HPA-axis in stress regulation and the pathogenesis of depression.

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