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Variation in the Glucocorticoid Receptor Gene at rs41423247 Moderates the Effect of Prenatal Maternal Psychological Symptoms on Child Cortisol Reactivity and Behavior

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Prenatal maternal psychopathology affects child development, but some children seem more vulnerable than others. Genetic variance in hypothalamic–pituitary–adrenal axis genes may influence the effect of prenatal maternal psychological symptoms on child emotional and behavioral problems. This hypothesis was tested in the Generation R Study, a population-based cohort from fetal life onward. In total, 1727 children of Northern European descent and their mothers participated in this study and were genotyped for variants in the glucocorticoid receptor (*GR*) gene (rs6189/rs6190, rs10052957, rs41423247, rs6195, and rs6198) and the *FK506-binding protein 5* (*FKBP5*) gene (rs1360780). Prenatal maternal psychological symptoms were assessed at 20 weeks pregnancy and child behavior was assessed by both parents at 3 years. In a subsample of 331 children, data about cortisol reactivity were available. Based on power calculations, only those genetic variants with sufficient minor allele frequencies (rs41423247, rs10052957, and rs1360780) were included in the interaction analyses. We found that variation in *GR* at rs41423247 moderates the effect of prenatal maternal psychological symptoms on child emotional and behavioral problems (beta 0.41, SE 0.16, $p = 0.009$). This prenatal interaction effect was independent of mother's genotype and maternal postnatal psychopathology, and not found for prenatal psychological symptoms of the father. Moreover, the interaction between rs41423247 and prenatal psychological symptoms was also associated with decreased child cortisol reactivity (beta −2.30, *p*-value 0.05). These findings emphasize the potential effect of prenatal gene–environment interaction, and give insight in possible mechanisms accounting for children's individual vulnerability to develop emotional and behavioral problems.
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INTRODUCTION

Maternal psychopathology during pregnancy has been associated with a broad range of temperamental difficulties and emotional and behavioral problems in children, such as increased anxiety, poorer attention, and hyperactivity (Van den Bergh *et al*, 2005), but the mechanisms accounting for these associations are only partly understood. It has been posited that in utero-exposure to stress increases fetal exposure to cortisol, which might influence the development of the fetal hypothalamic–pituitary–adrenal axis (HPA-axis) (Huizink *et al*, 2004; Seckl and Holmes, 2007; Weinstock, 2008). Indeed, mounting evidence points to an effect of maternal depression, anxiety, and stress during pregnancy on maternal cortisol levels, which in turn have been associated with increased cortisol levels and disrupted behavior in the offspring (Davis *et al*, 2007, 2011; Lundy *et al*, 1999; Wadhwa *et al*, 1996). Diathesis-stress models postulate that adversity in fetal life alters the development of neuronal and endocrine responses to stressors and predisposes individuals to disease (Entringer *et al*, 2010).

Yet, not every child of mothers with psychological symptoms during pregnancy will actually develop

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emotional and behavioral problems (Glover, 2011). This apparent individual vulnerability to the effect of maternal psychological symptoms may be partly explained by common variation in genes that regulate the HPA-axis. Prenatal gene–environment interaction is very plausible, but the empirical evidence is scarce (Glover, 2011). The aim of this study was to examine whether HPA-axis-related genes moderate the association between prenatal maternal psychological symptoms and child emotional and behavioral problems.

The HPA-axis is the main neuro-endocrine system that is activated in response to stress. The axis consists of the hypothalamus, the anterior pituitary, and the adrenal cortex. Glucocorticoids, that is cortisol, are the final effectors of the axis and exert a negative feedback effect on both corticotropin-releasing hormone (CRH) and adreno-corticotropic hormone production and secretion in the hypothalamus and the pituitary. In response to various physical and psychological stressors, the HPA-axis becomes activated and as a result the cortisol level increases. Cortisol exerts its effect via the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). In the brain, MR mediates the onset of the stress response, whereas GR is involved in the termination of the stress response (de Kloet *et al*, 2005). Within the GR gene (*GR*, *NR3C1*) region, several single-nucleotide polymorphisms (SNPs) (rs6189/rs6190, rs10052957, rs41423247, rs6195, and rs6198) have been studied in relation to HPA-axis function and psychiatric disorders. Associations have been found between *GR* SNPs and receptor sensitivity to cortisol (rs41423247, rs6195, rs6198, and rs6189/rs6190), hippocampal and amygdala size (rs10052957), and psychiatric disorders, such as depression (rs41423247 and rs6189/rs6190) and bipolar disorder (rs10052957) (Manenschijn *et al*, 2009). The sensitivity of the GR to cortisol is further influenced by the FK506-binding protein 5 (*FKBP5*), which acts as co-chaperone of *GR*. *FKBP5* SNPs (eg, rs1360780) have been associated with insufficient recovery of cortisol levels after psychosocial stress (Ising *et al*, 2008), with depression (Lekman *et al*, 2008; Zobel *et al*, 2010), and with the response to antidepressant treatment (Binder, 2009; Zou *et al*, 2010). Hence, these SNPs are attractive candidates to moderate the effect of prenatal maternal psychopathology on child development.

We hypothesized that vulnerability to prenatal maternal psychological symptoms is accentuated by common variation in *GR* and *FKBP5*, which may result in an increased risk for emotional and behavioral problems. This hypothesis was tested in 1727 children participating in a large population-based cohort. First, we evaluated the moderating effect of child SNPs in *GR* and *FKBP5* on the association between prenatal maternal psychological symptoms and child emotional and behavioral problems, controlling for maternal genotype, postnatal maternal psychological symptoms, and environmental factors. To study the plausibility of direct intra-uterine effects of maternal symptoms, we also evaluated the interaction between child SNPs and psychological symptoms of the father on child development. Second, in a subsample, we examined whether child SNPs interact with prenatal maternal psychological symptoms to influence child cortisol reactivity.

MATERIALS AND METHODS

Design

This study was embedded in the Generation R Study, a population-based cohort from fetal life onward in Rotterdam, The Netherlands. The Generation R Study has previously been described in detail (Jaddoe *et al*, 2007; Luijk *et al*, 2010). All children were born between April 2002 and January 2006. The study has been approved by the medical ethics committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all adult participants.

Population of Analysis

In genetic analyses, population stratification can increase the rate of false positive findings in heterogeneous samples like the Generation R Cohort. Hence, we selected children of Northern European descent, which was determined by principal component analyses of genome-wide association data, as described previously (Jaddoe *et al*, 2010). Principal component analyses yield factors that can be interpreted as the direction, which maximizes the variance of the sample while being uncorrelated to previous components. Within the children of Northern European descent ($n = 2650$), genetic data and information about prenatal maternal psychological symptoms were available in 2065 children. In 1727 (84%) of these children, data were available about child emotional and behavioral problems at the age of three years. These 1727 children comprised the population of analysis. Data on cortisol reactivity were available in 331 children participating in a subsample of children followed in more detail; the Generation R Focus Study (Jaddoe *et al*, 2010; Luijk *et al*, 2010).

Genotyping

DNA was collected from cord blood at birth. Participants were genotyped for six HPA-axis-related SNPs; rs6189/rs6190 (ER22/23EK), rs10052957 (TthIII1), rs41423247 (Bcl1), rs6198 (GR9beta), rs6195 (N363S), and rs1360780 (Derijk, 2009). These SNPs were chosen on the basis of their reported functionality (Geelhoed *et al*, 2010). Genotyping was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg, Germany). The genotyping reaction was amplified using the GeneAmp PCR system 9600 (95 °C, (15 min), then 40 cycles of 94 °C (15 s), and 60 °C (1 min)). The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems). Genotyping was successful in 97–99% of the samples. To confirm the accuracy of the genotyping, 276 randomly selected samples were genotyped for a second time with the same method. The error rate was $<1\%$ for all genotypes. Contamination with maternal blood occurred in $<1\%$ of cases. Mendelian errors occurred in $<0.5\%$ of cases; these were excluded. Allele frequencies were in Hardy–Weinberg equilibrium ($p > 0.05$).

Maternal Psychological Symptoms

Maternal and paternal psychological symptoms were assessed at 20 weeks of pregnancy and 2 months postnatal with the Brief Symptom Inventory, a validated self-report questionnaire with 53 items to be answered on a five-point scale, ranging from '0 = not at all' to '4 = extremely' (Beurs, 2004; Derogatis, 1993). The Global Severity Index (GSI) is the sum score of all 53 items and defines a broad spectrum of psychological symptoms (depression, hostility, anxiety, phobic anxiety, psychoticism, paranoid ideation, obsessive–compulsive, interpersonal sensitivity, and somatization). For prenatal psychological symptoms of the mother, the Cronbach's alpha was 0.91; for fathers it was 0.93. For maternal postnatal psychological symptoms alpha was 0.93.

Child Behavior

The child behavior checklist/$1\frac{1}{2}$–5 (CBCL/$1\frac{1}{2}$–5) was used to obtain standardized parent reports of children's emotional and behavioral problems at 3 years. This questionnaire contains 99 items, which are scored on a three-point scale: 0 = not true, 1 = somewhat true or sometimes true, and 2 = very true or often true, based on the two preceding months. The total problems score is obtained by summing the scores of all 99 items. Next to the total problems score, six syndrome scales are obtained; emotionally reactive, anxious/depressed, somatic complaints, and withdrawn, attention problems and aggressive behavior. The psychometric properties of the CBCL are well established (Achenbach and Rescorla, 2000). The alpha for the CBCL total problem scores as reported by the mother was 0.91; for the CBCL total problem scores as reported by the father alpha was 0.93.

Child Cortisol Reactivity

Cortisol reactivity was assessed in response to stress evoked by the strange situation procedure (SSP) (Ainsworth *et al*, 1978) at age 14 months, in line with other studies (Hertsgaard *et al*, 1995; Tollenaar *et al*, 2011). The SSP measures the quality of the attachment relationship and is a widely used and well-validated procedure to evoke mild stress in infants. The procedure consists of seven episodes of 3 min each in which stress is evoked by the unfamiliar lab environment, a female stranger entering the room and engaging with the infant, and the parent leaving the room twice (Luijk *et al*, 2010). To assess cortisol reactivity, three saliva samples were taken using Salivette sampling devices (Sarstedt, Rommelsdorf, and Germany); the first before the SSP, the second directly after the SSP, and the third 15 min later. Samples were centrifuged and frozen at $-80\,^{\circ}$C. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Germany). Intra- and interassay coefficients of variation were below 7% and 9%, respectively. For each time point, cortisol values above the 99th percentile (>200 nmol/l) were excluded ($n = 12$) from the analysis reducing the impact of outliers. For stress reactivity, a delta was calculated between the last sample and the first sample. To control for the Law of Initial Values (Wilder, 1968), which states that the direction of response of a body function depends to a large degree on the initial level; this delta was statistically adjusted for the first sample just before the stressful situation. This adjustment also controls for the different times of sampling.

Covariates

Gestational age was established by fetal ultrasound examinations. Information about Apgar score, birth weight, and gender of the infant was obtained at birth. Information about maternal age, educational level, smoking during pregnancy, parity, and child age was obtained by questionnaire. The inclusion of these potential confounders was primarily determined *a priori* and based on existing knowledge about the association between prenatal parental psychopathology and child development (Van den Bergh *et al*, 2005).

Statistical Analysis

Differences in baseline characteristics of responders ($n = 1727$) and non-responders ($n = 334$) to the CBCL/$1\frac{1}{2}$–5 were compared with the χ^2 statistic for categorical variables, the independent *t*-test for normally distributed continuous variables and the Mann–Whitney *U*-test for non-normally distributed continuous variables. The CBCL total problems scores of mother and father were square root transformed to achieve a normal distribution. Next, total problems scores and syndrome scores were z-standardized, summed and divided by two to obtain average scores based on both informants. Using the information of two raters strengthens the reliability of the outcome measure. Also, using a secondary informant reduced possible reporter bias because this information obtained of the father is less likely to be influenced by the prenatal report of the mother. If only the score of one parent was available, this score was used (12%). The syndrome scores could not be normalized and were analyzed as dichotomous variables. As Dutch norm scores have not been published, the 80th percentile was used as cut-off to differentiate between children with low and high scores on syndrome scales, in line with previous analyses (Velders *et al*, 2011). Missing values on maternal SNPs, Apgar score, and maternal psychological symptoms 2 months after child birth were imputed using a fully conditional specified model (maximum 12% missing). Pooled estimates from the five imputed data sets were used to report the effect estimates (beta's) and their standard errors (SE) (SPSS, PASW Statistics Rel 17.0.2 SPSS Chicago, 2009). Power analyses were performed using Quanto v1.2 (Gauderman, 2006). The decision to include SNPs in the analysis was based on the criterion that the estimated power was 0.80 or above. To rule out gene–environment correlation, we computed correlations between child SNPs and prenatal maternal psychological symptoms (Spearman, two-tailed).

First, we tested for genetic and environmental main effects. To test our hypothesis, we examined whether child SNPs moderate the effect of prenatal maternal psychological symptoms on child emotional and behavioral problems using multivariate linear regression. This model assumes additive genetic interaction, and optimizes statistical power. In these analyses, prenatal and postnatal psychological problems of the parents were used as continuous variables.

The final regression model testing the interaction between rs41423247 and prenatal maternal psychological symptoms included the following covariates: maternal SNPs, maternal age, maternal education, maternal smoking during pregnancy, parity, Apgar score 5-min postnatal, child gender, gestational age, postnatal maternal psychological symptoms, and child age at time of assessment behavior (SPSS, PASW Statistics Rel 17.0.2 SPSS Chicago, 2009).

Second, to explore the specificity of these interactions, we tested whether child SNPs moderated the association between *postnatal maternal psychological symptoms* and child emotional and behavioral problems. We also tested whether child SNPs moderated the association between *prenatal psychological symptoms of the father* and child development. In addition, we tested whether *maternal SNPs* also moderated the association between prenatal maternal psychological symptoms and child emotional and behavioral problems.

Third, we aimed to identify whether specific emotional and behavioral problems underlie the interactions and extended the findings to syndromes of emotional and behavioral problems. In these logistic regression analyses, multiplicative interaction is tested. For these analyses, we dichotomized the GSI on the 85th percentile to distinguish between mothers with and without prenatal psychological symptoms, in concordance with previous studies (van Batenburg-Eddes *et al*, 2009; van den Berg *et al*, 2009). To study deviation from additive interaction between two risk factors, we calculated the synergy index (S). S equals $[OR_{++}-1]/[(OR_{+-}-1)+(OR_{-+}-1)]$ and reflects the excess risk because of interaction relative to the risk from exposure to both determinants without interaction (de Mutsert *et al*, 2009). In the absence of interaction, S equals 1. Finally, we explored the possible biological impact of the interaction between child SNPs and prenatal maternal psychological symptoms by focusing on a child cortisol reactivity. In these analyses, we used the dichotomized measure of prenatal maternal psychological symptoms, because in this subsample the residuals were no longer normally distributed if used as continuous measure.

Non-Response Analysis

Mothers who did not complete the CBCL/$1\frac{1}{2}$–5 ($n=326$) were on average younger (30.5 *vs* 32.1 years, $t=5.94$, $p<0.001$), were less likely highly educated (29.1% *vs* 42.5%, $\chi^2=20.39$ (1 df), $p<0.001$), and more likely to smoke during pregnancy (22.8% *vs* 19.15%, $\chi^2=30.72$ (1 df), $p<0.001$) than responding mothers ($n=1727$). Children of non-responding mothers had on average a lower birth weight (3478 *vs* 3583 g, $t=-3.50$, $p=0.001$). The full characteristics of mothers and children in our study sample are presented in Table 1.

RESULTS

Table 2 presents the distribution of SNPs. Minor allele frequencies (MAFs) ranged from 3 to 37%. Power calculations left three SNPs for analyses; rs10052957 (MAF 31%), rs41423247 (MAF 37%), and rs1360780 (MAF 29%). Rs10052957, rs41423247, and rs1360780 were not significantly correlated with prenatal maternal psychological symptoms (rs10052957 Spearman's $\rho=-0.016$, $p=0.503$; rs41423247 Spearman's ρ 0.009, $p=0.709$; rs1360780 Spearman's $\rho=-0.023$, $p=0.345$), which makes it less likely that gene–environment correlation was misinterpreted as interaction (Rutter *et al*, 2006). As presented in Table 3, regression analyses indicated a significant main effect of prenatal maternal psychological symptoms on child emotional and behavioral problems (beta 1.23, $p<0.001$), which was slightly attenuated after adjustment for covariates and postnatal maternal psychological symptoms (beta 0.91, SE 0.13, $p<0.001$). There were no genetic main effects.

Table 1 Maternal and Child Characteristics ($n=1727$)

	Mean (SD)[a]
Mother	
Age at child birth	32.1 (3.8)
Educational level (%)	
Higher education	42.5
Smoking during pregnancy (%)	
Never	80.9
Parity	
First born	60.1
Child	
Gender (% boys)	50.4
Birth weight	3583.0 (506)
Gestational age (weeks)	40.4 (29.9; 43.4)[b]
CBCL total problems score	18 (0; 69)[b]

Abbreviation: CBCL, child behavior checklist.
[a]Unless otherwise indicated.
[b]Median (100% range).

Of the three candidate SNPs, rs41423247 moderated the association between prenatal maternal psychological symptoms and child emotional and behavioral problem scores. The interaction remained significant after adjustment for mother's genotype and potential confounders (model 3; beta 0.41, $p=0.009$), and after correction for multiple testing ($p_{\text{Bonferroni}}$ $0.05/3=0.017$).

This dose–response effect of variation in *GR* at rs41423247 based on untransformed variables is displayed in Figure 1 and shows that in children of mothers with prenatal psychological symptoms the risk of emotional and behavioral problems increases in heterozygous carriers and homozygous carriers of the minor allele (Cytosine (C)) at rs41423247. For instance, homozygous carriers of the minor allele (CC) with mothers that had prenatal psychological symptoms scored higher on emotional and behavioral problems (CBCL/$1\frac{1}{2}$–5 total problems score 41 than exposed non-carriers (GG) (CBCL/$1\frac{1}{2}$–5 total problems score 17. This moderation by rs41423247 was not observed in children with mothers that had low prenatal psychological symptoms (CC CBCL/$1\frac{1}{2}$–5 total problems score 17 *vs* GG CBCL/$1\frac{1}{2}$–5 total problems score 18). The figure based on the square root transformed outcome measure is presented in the Supplementary material (Supplementary Figure S1).

Further evaluation of this prenatal G × E indicated that child rs41423247 neither interacted with *postnatal* maternal psychological symptoms ($n=1501$, beta 0.07, $p=0.64$) nor

Table 2 Distribution of Single-Nucleotide Polymorphisms Located in the *Glucocorticoid Receptor* Gene and the *FKBP5* Gene ($n = 1727$)

SNPs	Chr.	Variant	Minor allele number of copies			MAF	HWE *p*-value
			0	1	2		
Glucocorticoid receptor gene (GR/NR3C1)							
rs6189/6190	5	G→A	1614	97	0	3	0.228
rs10052957	5	G→A	787	739	159	31	0.444
rs41423247	5	G→C	675	790	243	37	0.628
rs6195	5	A→C	1542	135	5	4	0.254
rs6198	5	A→G	1144	478	39	16	0.185
FKBP5 gene							
rs1360780	6	C→T	843	730	130	29	0.103

Abbreviations: Chr., chromosome; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNPs, single-nucleotide polymorphism.

Table 3 Interaction Effect of HPA-Axis SNPs with Prenatal Maternal Psychological Symptoms on Child CBCL Total Problems Scores (sqrt) Parent Report at 3 Years ($n = 1727$)

	CBCL/1½-5 total problems score (sqrt) parent report at 3 years								
	Model 1 unadjusted			Model 2 adjusted for maternal genotype			Model 3 additionally adjusted for confounders[a]		
	Beta	SE	*p*-Value	Beta	SE	*p*-Value	Beta	SE	*p*-Value
Prenatal maternal psychological symptoms (E)	1.23	0.11	<0.001						
rs10052957	0.04	0.03	0.272						
rs10052957 × E	0.22	0.18	0.204	0.23	0.18	0.200	0.22	0.17	0.200
rs41423247	0.04	0.03	0.210						
rs41423247 × E	0.38	0.16	0.018	0.38	0.16	0.018	0.41	0.16	0.009
rs1360780	−0.01	0.03	0.710						
rs1360780 × E	0.31	0.18	0.078	0.31	0.18	0.079	0.27	0.18	0.124

[a]Model 3; adjusted for maternal genotype, maternal age, maternal education, maternal smoking during pregnancy, parity, Apgar score 5-min postnatal, child gender, gestational age, postnatal maternal stress, and child age at time of assessment behavior.

with prenatal psychological symptoms of *the father* ($n = 1463$, beta −0.15, $p = 0.53$) to influence the risk of child emotional and behavioral problems. Furthermore, *maternal genotype* at rs41423247 did not interact with prenatal psychological symptoms ($n = 1488$, beta 0.15, $p = 0.36$).

We extended this finding to syndromes of child problems. These analyses revealed that the interaction between rs41423247 and prenatal maternal psychological symptoms significantly increased the risk for aggressive behavior (aOR 1.76, 95% CI 1.12; 2.74, $p = 0.014$) and anxious/depressed behavior (aOR 1.71, 95% CI 1.07; 2.73, $p = 0.025$) (Supplementary Table S1). The synergy indices for these findings were 3.73 for the aggression syndrome and 2.94 for the anxious/depressed syndrome indicating deviation from additivity.

To evaluate the biological impact, we tested for the interaction between rs41423247 and prenatal maternal psychological symptoms on cortisol reactivity in 331 toddlers. We did not find main effects of rs41423247 (beta −0.13, SE 0.84, $p = 0.882$) and prenatal maternal psychological symptoms (beta −0.14, SE 0.42, $p = 0.743$) on cortisol reactivity. Moreover, rs41423247 interacted with prenatal maternal psychological symptoms to influence child cortisol reactivity after stress (beta −2.30, SE 118, *p*-value 0.053) (Figure 2). If the mother reported high prenatal maternal psychological symptoms, children with the CC genotype showed significantly less cortisol reactivity than children with the GG genotype (beta −2.00, SE 0.87, $p = 0.026$). *Post hoc* analyses revealed that child cortisol levels 15 min after the SSP accounted for the prenatal interaction effect on cortisol reactivity (beta −2.67, SE 1.32, $p = 0.044$) (Supplementary Table S2). The effect of the minor allele on cortisol reactivity was not found in children of mothers with low prenatal maternal psychological symptoms (beta 0.23, SE 0.47, $p = 0.629$).

DISCUSSION

This study investigated children's individual vulnerability to maternal psychological symptoms during pregnancy and the effect of this prenatal G × E on emotional and

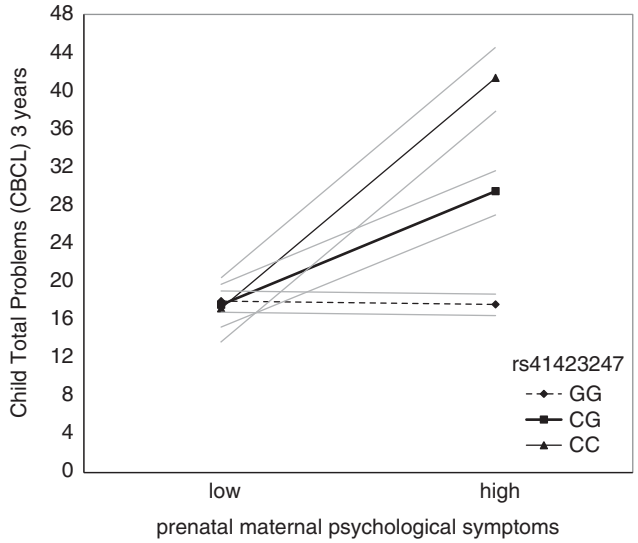

Figure 1 Results of adjusted linear regression estimating the association between low and high prenatal maternal psychological symptoms on child emotional and behavioral problems at age 3 as a function of variance in *GR* at rs41423247 ($n = 1704$). The main effect of variance at rs41423247 was not significant (beta 0.04, $p = 0.210$). The main effect of prenatal maternal psychological symptoms was significant (beta 1.23, $p < 0.001$) and the G × E interaction was in the expected direction (beta 0.38, $p = 0.018$). The interaction showed a dose-response effect of the minor allele at rs41423247 on the risk of emotional and behavioral problems in children of mothers with high scores on prenatal maternal psychological symptoms, which was absent in children of mothers reporting low on prenatal maternal psychological symptoms. The dotted lines represent the 95% confidence intervals around the effect estimates.

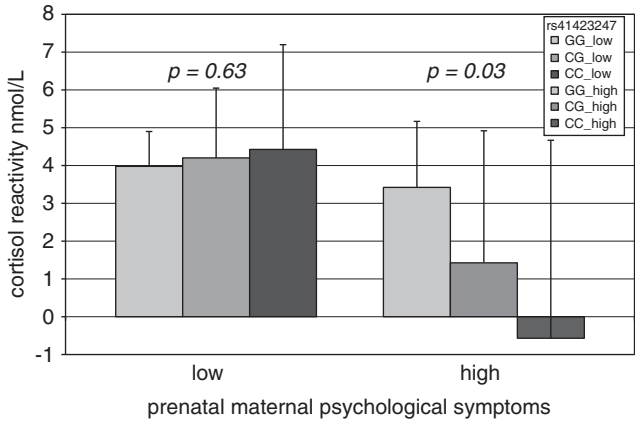

Figure 2 Results of linear regression analyses estimating the association between low (<85th%ile) and high (⩾85th%ile) prenatal maternal psychological symptoms and child cortisol reactivity after stress as a function of variance in *GR* at rs41423247. The main effect of variance at rs41423247 was not significant (beta −0.13, SE = 0.84, $p = 0.882$). The main effect of prenatal maternal psychological symptoms was not significant (beta −0.14, SE = 0.42, $p = 0.743$). The G × E interaction was in the expected direction (beta −2.30, SE = 1.18, $p = 0.05$). The minor allele at rs41423247 was associated with decreased cortisol reactivity in children of mothers with high prenatal maternal psychological symptoms (beta −2.00, SE 0.87, $p = 0.03$), which was absent in children of mothers reporting low prenatal maternal psychological symptoms (beta 0.23, SE 0.47, $p = 0.63$).

behavioral problems later in life. We found that a common variant in *GR* at rs41423247 (BclI) moderates the relation between prenatal maternal psychological symptoms and child emotional and behavioral problems. Maternal genotype at rs41423247 and postnatal maternal symptoms did not account for this effect. Also, the interaction between rs41423247 and prenatal symptoms of the father was not significant. Together, these findings seem to provide evidence for a prenatal G × E of child rs41423247 and prenatal maternal psychological symptoms, which influences the intra-uterine environment and results in an increased risk for emotional and behavioral problems in preschool children. Moreover, this prenatal gene–environment interaction may also affect HPA-axis function, as we found attenuated cortisol reactivity in response to stress in a subsample of 14-month-old carriers of the minor allele at rs41423247, but only if they had mothers with prenatal maternal psychological symptoms.

The SNP at rs41423247 is located in intron B, 647 bp downstream of exon 2, and results in a guanine to cytosine alteration (Derijk, 2009). The direction of the results of our study conform to previous research reporting an association between rs41423247 and increased sensitivity to glucocorticoids following a DEX-CRH test, lower cortisol response after psychological stress (Derijk, 2009; Manenschijn *et al*, 2009), and major depression disorder (Krishnamurthy *et al*, 2008; Kumsta *et al*, 2007). The mechanism by which this SNP exerts its effect remains unclear because it is not located in a coding, regulatory or splicing part of *GR*. Recently, it was shown that the minor allele is associated with decreased abundance of the predominant GRalpha receptor isoform in the dorsolateral prefrontal cortex, which results in abnormal GR expression (Sinclair *et al*, 2012).

Katz *et al* (2012) found increased expression of NR3C1 and FKBP5 during pregnancy. In depressed women, the increase in FKBP5 expression was smaller than in non-depressed women. There was no difference in NR3C1 expression between depressed and non-depressed women. Also, rs41423247 is located in a single linkage disequilibrium block with several other SNPs (Rautanen *et al*, 2006). Hence, rs41423247 could also be a marker of the actual functional variant, possibly located in the promoter region of *GR*. In this study, information about methylation status was not available. In rats, DNA methylation of the *GR* promoter region, which influence GR expression (Weaver *et al*, 2004), has been associated with the programming effect of maternal licking and grooming on the HPA-axis (Liu *et al*, 1997). Importantly, programming of the HPA-axis in rat occurs during the early postnatal period, whereas in humans neuroendocrine development takes place before birth (Weinstock, 2008). This could explain our finding that rs41423247 specifically moderates the effect of maternal psychological symptoms during pregnancy, and not after birth.

Maternal depressed mood during pregnancy has been associated with increased methylation of the *GR* promoter region in neonates, which is turn predicted increased HPA-axis reactivity in these neonates at 3 months (Oberlander *et al*, 2008). In contrast, chronic stress, like depression, is typically related to flattening of daytime cortisol rhythms and a blunted cortisol response, which has been described in children raised in neglectful environments (Gunnar and Vazquez, 2001). In the general population, parental

depression has been related to attenuated cortisol reactivity in response to stress in adolescents (Bouma *et al*, 2011). We showed that also in a population-based cohort of relatively healthy young children, the interaction between rs41423247 and prenatal maternal psychological symptoms resulted in an attenuated cortisol response. As cortisol reactivity at the age of 14 months and problem behavior at 3 years were not related we should be careful to infer a causal pathway. Hence, we can only speculate whether lower cortisol reactivity is more a global indication of HPA-axis vulnerability or part of the underlying pathophysiology of child problems. Importantly, it also indicates a consistency in results and makes a chance finding less likely.

There was no significant main effect of rs41423247 on the risk of child emotional and behavioral problems. Replicable genetic main effects are seldom found in psychiatric genetics (Rutter *et al*, 2009). This observation from linkage analyses and candidate gene studies is underscored by genome-wide association studies. Although expectations were high, the genome-wide approach has not yet found the genes accounting for the high heritability estimates of most psychiatric disorders obtained in twin studies. In search for this missing heritability, it has been posited that at least part of it must be hidden in gene–environment interactions (Uher, 2009). Gene–environment interactions are not conditional on the presence of main effects. If the genetic effect is only apparent in the high range of the environmental stressor and not in the low range, there may indeed be gene–environment interaction without a genetic main effect (Rutter *et al*, 2009; Uher and McGuffin, 2008).

Few studies reported G × E with *GR* SNPs. Bet *et al* (2009) reported an interaction of rs6189/rs6190 and rs6198 with childhood adversity on adult depression. Interactions have been reported for *FKBP5* SNPs with childhood abuse and risk for PTSD (Binder *et al*, 2008), and with infant attachment on cortisol reactivity in children (Luijk *et al*, 2010). To the best of our knowledge, this is the first study to report moderation by rs41423247.

There are several limitations to this study. First, even in this large population-based cohort, we did not have sufficient power to study all *a priori* selected candidate SNPs. So, we restricted the analyses to those SNPs with a MAF for which the present study yielded sufficient power. Second, observational measurements in this large cohort were not feasible. Therefore, we relied on report of parents on psychological problems and child behavior. Yet, we used validated questionnaires with good reliability and validity. Third, to reduce possible bias because of population heterogeneity only children of Northern European descent were included in the analyses. Therefore, we should be careful generalizing our findings to other populations. Fourth, information about child cortisol levels was only available in a subsample. Currently, saliva samples are being collected in the children at the age of 5. In the future, these samples will provide us with information about cortisol daily rhythms in a much larger sample. This will enable us to further study the mechanisms underlying the interaction of child allelic variants with prenatal maternal psychological symptoms and HPA-axis regulation.

In conclusion, we found evidence for prenatal gene–environment interaction of *GR* rs41423247 with maternal psychological symptoms resulting in an increased risk of child emotional and behavioral problems. This interaction also seems to affect child cortisol reactivity. These findings emphasize the potential effect of prenatal programming on child development, and give further insight in possible mechanisms accounting for the differences in children's vulnerability to maternal psychological symptoms.

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DISCLOSURE

The authors declare no conflict of interest. Professor Dr FCV is a contributing author of the Achenbach System of Emperically Based Assessments, from which he receives remuneration.

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