Infant attachment and stress regulation : a neurobiological study

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3 FKBP5 and resistant attachment predict cortisol reactivity in infants: Gene-environment interaction

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Abstract

Quality of the parent-infant attachment relationship influences physiological stress regulation. Genetic factors also contribute to the stress regulatory HPA-axis. Quality of attachment as an index of the rearing environment (measured with the Strange Situation Procedure, SSP), and HPA-axis related SNPs (*Bcl*I, rs41423247; *TthIII*I, rs10052957; GR-9β, rs6198; N363S, rs6195; ER22/23EK, rs6189 and 6190; and FKBP5, rs1360780) were hypothesized to be related to cortisol reactivity in the stressful SSP. In this large population based sample, FKBP5 rs1360780, but not GR haplotype, was related to cortisol reactivity. Moreover, we found a significant interaction effect for insecure-resistant attachment and FKBP5 rs1360780, indicating a double risk for heightened cortisol reactivity levels in infants with one or two T-alleles of the FKBP5 SNP *and* an insecure-resistant attachment relationship with their mother. Findings are discussed from the perspective of gene-environment interaction.

Introduction

The infant-parent attachment relationship plays a major role in the infant's early life, particularly for socio-emotional development and emotion regulation (Bowlby, 1969/1982; Cassidy, 1994). The quality of the attachment relationship not only influences regulation on the behavioral level, but also affects physiological regulation. The physiological system is activated in stressful contexts, especially when coping behaviors are inadequate or coping resources are unavailable (Hertsgaard et al., 1995). Most studies on the physiology of infant attachment relationships focused on measures of heart rate and cortisol during the Strange Situation Procedure (SSP, Ainsworth et al., 1978; e.g. Gunnar et al., 1989; Oosterman & Schuengel, 2007; Sroufe & Waters, 1977), a mildly stressful procedure with two brief separations from the caregiver in an unfamiliar environment. Differences

in physiology during this procedure have been predominantly attributed to the quality of attachment which is an index of the rearing environment. Genetic factors have not received much attention, although there is ample evidence that genetic factors play a role in explaining variance in HPA-axis activity (Bartels et al., 2003; Steptoe et al., 2009; Wüst et al., 2004a). In the current study, both quality of attachment and genetic variations associated with HPA-axis activity were examined in relation to cortisol reactivity. In addition, the interaction between genetic factors and attachment quality on cortisol reactivity was investigated.

Studies on the association between attachment quality and cortisol levels have focused mainly on stress reactivity, with assessment of cortisol levels before and after the stressful SSP. The SSP is the gold-standard procedure to assess the quality of the infant-caregiver attachment relationship. The SSP allows for classification of the relationship as secure, insecure-avoidant, or insecure-resistant. Securely attached (B) children seek contact with the parent upon reunion, either physically or by distance interaction, to be comforted or reassured after the separation and resume exploration of the environment when they are settled. Based on their interactions with the caregiver, they have learned that she/he is available in times of stress. In contrast, infants of inconsistently sensitive or consistently insensitive parents do not come to expect their parents to be available in stressful situations, with insecure (avoidant or resistant) attachment relationships as a result. Children with insecure-avoidant (A) attachments focus on the environment at the moment of reunion, ignoring the parent or even turning away from them. The reunion behavior of an insecure-resistant (C) child is characterized by anxious contact seeking and clinging and at the same time resisting contact with the parent. On top of these classifications, attachment disorganization can be observed and rated. Disorganized (D) children show a temporary breakdown of their secure, avoidant or resistant strategy of dealing with the return of the parent after separation (Main & Solomon, 1990).

In several non-clinical studies, children tended to show elevated cortisol levels in reaction to the SSP. The most consistent finding is that only little or no adrenocortical activation is observed in securely attached infants, and increased cortisol levels for the disorganized infants (Gunnar et al., 1996; Hertsgaard et al., 1995; Spangler & Grossmann, 1993). Results for the insecure-avoidant and insecure-resistant groups are inconsistent. In some studies, both insecure groups were found to have elevated cortisol levels after the SSP (Spangler & Grossmann, 1993), others found increased cortisol levels only for insecure-resistant children (Spangler & Schieche, 1998). In a previous study on the current sample (Luijk et al., 2010) we found increased cortisol levels for insecure-resistant children.

Associations between attachment quality and cortisol reactivity implicate that cortisol reactivity levels are, at least partly, determined by the caregiving environment (Gunnar & Quevedo, 2007; Meaney, 2001). Evidence for the contribution of genetic factors has been mixed (Ouellet-Morin et al., 2008;

Steptoe et al., 2009; Wüst et al., 2004a), and it has been noted that 'the genetic and environmental contributions to cortisol reactivity in early childhood have yet to be documented' (Ouellet-Morin et al., 2008, p. 212). This interplay between genetic and environmental factors was recently studied by Frigerio and colleagues (2009), who found independent effects of candidate genes (5HTT, GABRA6, DRD4, and COMT) and attachment quality on alpha amylase, another potential biomarker for physiological arousal. They did not, however, find effects of attachment quality, genetics, or their interaction on cortisol reactivity. In view of these diverging findings, they note that replication in larger samples is required.

Recently, specific candidate genes that play a role in explaining variability in cortisol reactivity have been identified. Several studies focused on the glucocorticoid receptor (GR) that mediates many of the effects of glucocorticoids. Genetic variants of the GR gene, e.g. single nucleotide polymorphisms (SNPs), appear to contribute to interindividual variability in HPA-axis activity by affecting a cell's sensitivity for glucocorticoids (DeRijk & De Kloet, 2008; Wüst et al., 2004b). Five different SNPs within the GR have been investigated in previous research; *Bcl*I (rs41423247), *TthIII*I (rs10052957), GR-9β (rs6198), N363S (rs6195) and ER22/23EK (rs6189 and 6190). No effects of GR on basal cortisol excretion have been found (Rautanen et al., 2006; Rosmond et al., 2000). However, HPA-axis reactivity as assessed using a social stressor, the Trier Social Stress Test, showed that carriers of the N363S G allele had increased cortisol responses. On the other hand, carriers of the *Bcl*I G allele and GR-9β G allele showed an attenuated response (Ising et al. 2008; Kumsta et al., 2007; Wüst et al., 2004b).

Importantly, blocks of specific SNP combinations are usually found within genes, resulting in several haplotypes, that is, groups of specific SNPs in a gene that tend to be inherited together. These haplotypes can have different effects compared to 'isolated' SNPs (DeRijk et al., 2008). In the current study, effects of GR haplotypes on cortisol reactivity will be tested (Van den Akker et al., 2006; 2008). GR activation is regulated by a large molecular complex. In this complex, several molecules, so-called chaperones and co-chaperones, play a critical role. Altering the composition of the (co-)chaperones influences sensitivity of GR to cortisol and thus affects HPA-axis responsivity (Binder et al., 2004; Binder, 2009). The FKBP5 co-chaperone of GR has been associated with changes in HPA-axis activity by altering the negative feedback system (Ising et al., 2008). The feedback loop is crucial in recovery from stress, which in turn is essential for healthy physiological and behavioral regulation. As the infant-parent attachment relationship can be considered the infant's most important emotion regulation system (Bowlby, 1969/1982, Cassidy, 1994), the role of a genetic factor influencing homeostasis might be of great importance. FKBP5 has several SNPs, and for these SNPs the most consistent findings were reported for rs1360780. For individuals carrying one or two copies of the minor (T) allele, i.e. the allele that is less frequent in the population, positive associations have been found with major depression, bipolar

disorder, post-traumatic stress disorder and a faster response to antidepressant treatment (for a review, see Binder, 2009). With respect to HPA-axis activity, this SNP did not show an effect on basal cortisol levels (Binder et al., 2004), but it did show an effect on cortisol responses to the Trier Social Stress Test (Ising et al., 2008). Participants who were homozygous for the minor allele (TT genotype) showed an impaired recovery from stress compared to carriers of the CC or CT genotype.

In the current study we expand the findings on attachment security and cortisol reactivity from previous studies (Gunnar et al., 1996; Hertsgaard et al., 1995; Spangler & Schieche, 1998) by adding a genetic component. Carriers of the minor alleles of the haplotypes of GR and the FKBP5 SNP were expected to show altered cortisol reactivity levels. Furthermore, it is hypothesized that the association between attachment security and stress reactivity is moderated by GR and FKBP5. A combination of insecure-resistant attachment and carrying one or more 'risk alleles' of GR and the FKBP5 SNP was expected to lead to higher cortisol reactivity.

Method

Setting

The current investigation is embedded within the Generation R Study, a prospective cohort study investigating growth, development and health from fetal life into young adulthood in Rotterdam, The Netherlands, which has been described in detail elsewhere (Jaddoe et al., 2007; 2008). In the Generation R Study, we obtained detailed measurements of the child's development in a rather homogeneous subgroup: The Generation R Focus Study. Only children of Dutch national origin were included in this group, meaning that the children, their parents and their grandparents were all born in the Netherlands. The participating children were born between February 2003 and August 2005. The children visited the research center regularly for various somatic and behavioral assessments. Written informed consent was obtained from all participants. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam.

Study population

DNA was collected from cord blood samples at birth. At the age of 14 months, infants and their mothers participated in the Strange Situation Procedure (SSP). In 589 infants, information on GR and FKBP5 genotypes and quality of attachment was available. Of this group, cortisol was sampled in 310 children. Unsuccessful sampling was mainly due to refusal to chew on cotton swabs, which is not uncommon in this age group and has been reported before (Goldberg et al., 2003). This is typically found in infants that are not familiar with pacifiers. A non-response analysis was conducted to check for differences between children with and without cortisol data. Differences between the groups were found for gender

(p = .01); the group with cortisol data consisted of more boys. Also, educational level of the mother differed (p = .03); mothers in the group for which data was available were less highly educated. No differences were found in the distribution of attachment classifications or genotype (.09 < p < .87).

Procedures and measures

Strange Situation Procedure. Parent-infant dyads were observed in the Strange Situation Procedure (SSP, Ainsworth et al., 1978) when the infant was about 14 months of age (M = 14.7, SD = 0.9). The SSP is a widely used and well-validated procedure to measure the quality of the attachment relationship. The procedure consists of seven episodes of 3 minutes each and is designed to evoke mild stress in the infant to trigger attachment behavior evoked by the unfamiliar lab environment, a female stranger entering the room and engaging with the infant, and the parent leaving the room twice (see Ainsworth et al., 1978, for the protocol). The SSP used in the current study included all these stimuli but to make it fit into a tight time schedule, we shortened the (pre-) separation episodes with one minute keeping the critical reunion episodes intact. Attachment behavior was coded from DVD-recordings according to the Ainsworth et al. (1978) and Main and Solomon (1990) coding systems by two reliable coders, trained at the University of Minnesota. Inter-coder agreement was calculated on 70 SSPs that were coded by both coders. For ABCD classification, inter-coder agreement was 77% (κ = .63); agreement on disorganization was 87% (κ = .64). Eight percent of the cases were discussed with one of two expert coders and classification was assigned after consensus was reached. We also coded the percentage of time the infant was crying during the SSP (intercoder reliability ICC = .98) to include as a covariate in the analyses.

Salivary cortisol: stress reactivity. During the visit at the research centre at 14 months of age, three saliva samples were taken using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany); the first prior to the SSP, the second directly after the SSP (which was on average 10 minutes after the first separation of the SSP) and the third about 15 minutes later (M = 16.3, SD = 8.3). None of the children used systemic corticosteroid medication, but 12 children used other corticosteroid-containing medication. Excluding these children did not change the results, thus we included them in further analyses.

Samples were centrifuged and frozen at -80°C. After completion of the data collection, all samples were sent in one batch (frozen, by courier) to the Kirschbaum laboratory (Technical University of Dresden, Biological Psychology, Professor Dr. Kirschbaum) for analysis. 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 that were above the 99th percentile (>200 nmol/L) were excluded (n = 12) from the analysis to reduce the impact of outliers.

Cortisol analyses. For stress reactivity a delta was calculated between the last sample ($cortisol_{postSSP}$) and the first sample ($cortisol_{preSSP}$). The second assessment, just after the SSP, was not used, as it was too close to the onset of stress. To control for the Law of Initial Values (LIV; Wilder, 1968), which states that the direction of response of a body function depends to a large degree on the initial level of that function, in subsequent analyses this delta was adjusted for the first sample.

Genotyping. DNA was collected from cord blood samples at birth. To check for potential contamination with maternal blood, gender was determined in male participants. Contamination occurred in < 1% of cases, which were excluded. All participants were genotyped for polymorphisms in the glucocorticoid receptor gene, *Bcl*I (rs41423247), *TthIII*I (rs10052957), GR-9β (rs6198), N363S (rs6195) and ER22/23EK (rs6189 and 6190); and the FKBP5 gene (rs1360780). Table 1 shows the allele frequencies for the GR SNPs. 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 results 276 randomly selected samples were genotyped for a second time with the same method. The error rate was less than 1% for all genotypes. For the glucocorticoid receptor gene we used the genotype data for each of the 5 polymorphisms to infer the haplotypes present in the population using the program PHASE, which implements a Bayesian statistical method for reconstructing haplotypes from population genotype data (Stephens et al., 2001). For each haplotype, three genotype combinations were distinguished as carrying 0, 1, or 2 copies of the haplotype allele. Haplotype 1 carries the major alleles of the polymorphisms; therefore, the reference allele is defined as carrying 2 copies of haplotype 1. The FKBP5 SNP was presented in a similar way; frequency of the minor allele was indicated (0, 1 or 2 copies). Distribution for FKBP5 was as follows: 147 CC (47.4%), 139 CT (44.8%), 24 TT (7.7%). Table 2 shows the specific nucleotide variations and distribution of the GR haplotypes and FKBP5. Genotype frequencies were in Hardy Weinberg equilibrium (χ^2s [1, N = 310] < 1.28, ps > .26). GR haplotypes and the FKBP5 SNP were not correlated.

Table 1. *Allele frequencies and minor allele frequencies for GR SNPs*

GR SNP	Allele frequency (%)[a]			MAF (%)
	0	1	2	
*Bcl*I (rs4142347)	41	46	13	36
*TthIII*I (rs10052957)	46	44	10	32
GR-9β (rs6198)	68	29	3	18
N363S (rs6195)	91	9	0	4
ER22/23EK (rs6189/6190)	92	8	0	4

[a] % of copies of the minor allele. MAF = minor allele frequency. All GR SNPs were in HWE (χ^2s [1, N = 310] < 0.66, ps > .42).

Statistical analyses. First, we checked whether demographic variables were related to cortisol, genotype, and attachment classification using ANOVAs and Chi-square tests. An ANCOVA was performed to test the association between attachment quality and cortisol reactivity levels, controlling for initial cortisol values. Because attachment security and attachment disorganization are considered orthogonal constructs (Van IJzendoorn et al., 1999), they were entered as two separate factors. The relation between attachment quality and genotypes was tested using a Chi-square test. A regression analysis was used to test the main effects of genotypes on cortisol reactivity, correcting for initial cortisol values. Associations were also tested for individual GR SNPs, which yielded similar results (data available upon request). Using a regression analysis, we tested for a potential interaction effect of insecure-resistant attachment, FKBP5, and GR on cortisol stress reactivity. For this analysis, infants with an insecure-resistant attachment classification were contrasted to infants with a non-resistant classification. In the first step, the first cortisol assessment ($cortisol_{preSSP}$) was entered to control for initial values. In the second step, resistant versus non-resistant attachment classification was entered. In the third step, GR haplotypes and FKBP5 were entered. In the fourth step, interactions between GR haplotypes, and FKBP5 with resistant attachment classification were entered. Except for the first cortisol assessment, all variables were centered based on the N for which cortisol reactivity data was available.

Table 2. *Distribution of GR haplotypes and FKBP5 and main effects on cortisol reactivity*

GR haplotype	Nucleotide variations					Haplotype copies (%)			HF (%)	*B* (95% *CI*)	*p* value
						0	1	2			
Wildtype	G	GG	A	C	A	33	49	18	42	0.12 (-4.29; 4.54)	.96
*Bcl*I	G	GG	A	**G**	A	62	33	5	22	0.49 (-3.98; 4.97)	.83
*TthIII*I + *Bcl*I	**A**	GG	A	**G**	A	72	27	1	14	0.68 (-3.89; 5.24)	.77
GR-9β + *TthIII*I	**A**	GG	A	C	**G**	75	24	1	13	1.07 (-3.49; 5.64)	.64
N363S	G	GG	**G**	C	A	91	9	0	4	1.46 (-3.51; 6.43)	.56
ER22/23EK + GR-9β + *TthIII*I	**A**	**AA**	A	C	**G**	92	8	0	4	-0.36 (-5.37; 4.66)	.89
FKBP5						Genotype frequency (%)			MAF (%)		
						CC	CT	TT			
rs1360780					C/T	47	45	8	30	1.28 (0.31; 2.25)	.01

Note. The nucleic acid changes are indicated in bold; C = Cytidine, G = Guanine, A = Adenine, T = Thymine. FKBP5 SNP was in HWE (χ^2 [1, N = 310] = 1.28, p = .26). MAF = minor allele frequency, HF = haplotype frequency.

Results

Distribution of attachment

In the group for which both cortisol reactivity data was available (N = 310), distribution of attachment classifications was as follows: 56.7% secure (n = 174), 18.6% insecure-avoidant (n = 57), 24.8% insecure-resistant (n = 76). Of all children, 18.4% were classified as disorganized (n = 57), 81.6% were non-disorganized (n = 253), a common distribution in non-clinical populations (Van IJzendoorn et al., 1999). Participant characteristics are displayed in Table 3. Time of cortisol assessment was not related to cortisol measures or attachment classification, in fact, none of the demographic variables were related to cortisol, genotype, and attachment classification at the same time.

Table 3. *Sample characteristics (N=310)*

Child characteristics	
Child gender, % girls	43.5
Parity, % firstborn	61.9
Birth weight in grams	3543 (483)
Age at 14 months visit	14.6 (0.8)
Time of assessment cortisol prior to SSP	12:07 (2:00)
Parental characteristics	
Age at intake mother	31.7 (4.1)
Maternal educational level, % low/medium	39.9
Hours working, mother	28.2 (12.9)
Marital status, % single	5.6
Smoking during pregnancy, %	11.0
Alcohol during pregnancy, %	55.2
Breastfeeding at 6 months, %	31.0

Note. Unless otherwise indicated, values are mean (*SD*).

Attachment quality, HPA-axis genes and cortisol reactivity

Infants with an insecure-resistant attachment relationship showed the highest cortisol reactivity levels from pre SSP to post SSP (F (2, 300) = 17.60, $p < .01$, η^2 = .11, see Table 4). We did not find significant differences in stress reactivity between the disorganized group and the non-disorganized group, nor an interaction effect of attachment security and attachment disorganization. Attachment quality was not related to glucocorticoid haplotypes ($.25 < p < .97$) or the FKBP5 SNP (rs1360780) (p = .14). Haplotypes of the glucocorticoid receptor were not related to cortisol reactivity ($.56 < p < .96$). The FKBP5 SNP was however related to cortisol reactivity (B = .13, CI = 0.31; 2.25, p = .01, see Table 1), indicating that infants with FKBP5-CT and TT genotypes showed increased cortisol reactivity.

The more T alleles infants carried, the stronger was their cortisol reactivity. Using a regression analysis, we tested for a potential interaction effect of insecure-resistant attachment, FKBP5, and GR haplotypes on cortisol stress reactivity. Main effects of GR haplotypes ($.14 < p < .91$) and interactions between GR haplotypes and resistant attachment did not reach significance ($.32 < p < .85$).

Table 4 . *Cortisol values pre SSP, post SSP and cortisol reactivity Δ (nmol/L)*

	Cortisol pre SSP	Cortisol post SSP	Cortisol reactivity Δ[a]	
Secure	6.26 (5.06)	6.15 (4.25)	-1.21 (0.87)	
Insecure-avoidant	6.27 (5.04)	5.42 (3.60)	-0.61 (0.62)	
Insecure-resistant	5.89 (4.97)	9.92 (8.91)	4.04 (0.65)	**
Disorganized	6.27 (7.02)	7.08 (8.09)	1.07 (0.37)	
Non-disorganized	6.15 (4.44)	6.89 (5.29)	0.41 (0.74)	

Note. Unless otherwise indicated, values are M (SD). Cortisol reactivity Δ corrected for initial cortisol values. [a] Values are M (SE). ** F (2, 300) = 17.60, $p < .01$

In Table 5, the most parsimonious model is presented. Main effects for resistant attachment and FKBP5 were significant (β = .30, $p < .001$; β = .19, $p < .001$, respectively), as was the interaction between FKBP5 and resistant attachment (β = .12, $p < .05$). The model explained 32% of the variance. Infants with a resistant attachment relationship and the FKBP5-CT genotype showed more increased cortisol reactivity than resistant infants with the CC genotype, and resistant infants with the FKBP5-TT genotype showed the largest increases in cortisol reactivity. The results remained essentially the same when infant crying during the SSP was included as a covariate.

Table 5. *Regression analysis predicting cortisol reactivity from FKBP5 and insecure-resistant attachment, controlling for initial cortisol values*

	B (95% *CI*)	β	*p*	F_{change}	R^2	R^2_{change}
Step 1				75.48	0.20	0.20
Cortisol$_{preSSP}$	-0.51 (-0.63;-0.40)	-0.42	<.001			
Step 2				36.52	0.28	0.09
Resistant attachment	2.10 (1.44; 2.76)	0.30	<.001			
Step 3				8.10	0.30	0.02
FKBP5	1.88 (0.85; 2.90)	0.19	<.001			
Step 4				5.29	0.32	0.01
FKBP5 * Resistant attachment	1.20 (0.17; 2.22)	0.12	.022			

Note. $R^2 = .32$. Final model F (4, 302) = 34.72, $p < .01$. β is a standardized coefficient and denotes SD change in cortisol reactivity per SD change in the predictor. The statistics are derived from the final block of the regression model.

Discussion

An insecure-resistant attachment relationship predisposes infants to heightened cortisol reactivity levels. Also, the minor allele of the FKBP5 SNP was associated with cortisol reactivity in an additive fashion; the more T alleles, the higher levels of cortisol reactivity. Furthermore, an interaction between insecure-resistant attachment and FKBP5 was found. This represents a double risk for heightened cortisol reactivity levels in infants who carry one or two T-alleles of the FKBP5 SNP and at the same time have an insecure-resistant attachment relationship with their mother.

Insecure-resistantly attached infants have been found to display high cortisol levels after a stressful stimulus in some studies (Spangler & Schieche, 1998), but not in others (Gunnar et al., 1989; Nachmias et al., 1996). Resistant infants' high activation of the attachment system may not be terminated soon after the reunion with the caregiver because they are unable to use the attachment figure effectively, which makes it difficult for these children to find a state of homeostasis (Cassidy & Berlin, 1994). In the current study, no effects of disorganization on stress reactivity were found. In a previous study on the same sample (Luijk et al., 2010), we found evidence for an association between disorganized attachment and flattened cortisol diurnal rhythm, which may indicate a different stress mechanism in the disorganized group. As determinants of attachment disorganization differ from those of attachment security, genetic susceptibility as well as physiological and behavioral developmental outcomes might be only partly overlapping.

FKBP5 rs1360780 has been associated with altered stress reactivity in an adult sample (Ising et al., 2008); individuals homozygous for the minor allele (TT genotype) showed an impaired recovery from stress. Furthermore, Binder et al. (2008) found that the FKBP5 SNP moderated the relation between child abuse and adult post-traumatic stress disorder (PTSD), and alterations in attachment quality or HPA-axis sensitivity were suggested as possible mechanisms for this association. Combined with findings from the current study, evidence grows for the contribution of this minor allele to differences in GR sensitivity, and to differential activation of the feedback loop of the HPA-axis when confronted by a psychological stressor (Binder, 2009). The negative feedback system is essential in recovery from stressful situations, for example the SSP. A balanced stress recovery system that promotes homeostasis is of great importance. Presumably, both the minor allele of the FKBP5 SNP and an insecure-resistant attachment relationship prevent adequate termination of the stress reaction. This lack of homeostasis could put the child at developmental risk; long-term negative outcomes have been shown for both insecure attachment (Fearon et al., 2010; Warren et al., 1997) and, indirectly, for carriers of the FKBP5 SNP (Binder et al., 2008). It should be noted however, that associations in the current study are correlational, and that the underlying mechanisms need further elaboration.

In the current study GR haplotypes were not related to cortisol reactivity. However, these results should be interpreted with some caution. Whereas we had sufficient power to analyze the two most frequent haplotypes, the haplotypes including the SNPs N363S and ER22/23EK displayed very low frequencies in the current sample. Few studies have investigated the association between GR haplotypes and cortisol reactivity, and report consistent but small effects of GR SNPs in adults (for a review, see Wüst et al., 2004a). In infants, these associations have remained largely uncharted. GR haplotypes and FKBP5 were not related to attachment security or attachment disorganization. This is consistent with findings from previous studies, as main effects of candidate genes on attachment quality have not been reliably established (Bakermans-Kranenburg & Van IJzendoorn, 2007). Recently, Frigerio and colleagues (2009) examined effects of attachment quality and candidate genes on alpha amylase and cortisol. They reported gene-environment interaction effects for alpha amylase, but no effects for cortisol. The current study investigated specific HPA-axis related genes in a large, population based sample, and provides evidence for effects of FKBP5, attachment quality and their interaction on cortisol reactivity.

The findings from the present study support the idea of interplay between genetic and environmental factors in explaining developmental outcomes (Rutter et al., 2006). Resistant attachment and FKBP5 predispose infants to increased cortisol reactivity both independently as well as in interaction. The current outcomes provide support for a double-risk model (Belsky et al., 2007) as the combination of environmental (indexed by resistant attachment) and genetic (FKBP5) risks increase stress reactivity in an additive way, even in a rather homogeneous, low-risk sample. In a more diverse sample the gene-environment interaction effect might even be larger. Furthermore, it should be noted that careful assessment of the environment is essential for establishing G x E interactions. In the current study, the quality of the attachment relationship offers an observation-based but indirect index of the environment. Detailed direct observation of parenting quality in the natural setting may offer a more complete assessment, but was beyond the scope of the current study.

In sum, the present study shows HPA-axis related genes and attachment quality to be associated with stress reactivity both independently and in interaction. The combination of an insecure-resistant attachment relationship and carrying the minor allele of the FKBP5 gene is related to increased stress reactivity in infants.