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Disentangling the effects of genetic, prenatal and parenting influences on children's cortisol variability

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Abstract

Developmental plasticity models hypothesize the role of genetic and prenatal environmental influences on the development of the hypothalamic–pituitary–adrenal (HPA) axis and highlight that genes and the prenatal environment may moderate early postnatal environmental influences on HPA functioning. This article examines the interplay of genetic, prenatal and parenting influences across the first 4.5 years of life on a novel index of children's cortisol variability. Repeated measures data were obtained from 134 adoption-linked families, adopted children and both their adoptive parents and birth mothers, who participated in a longitudinal, prospective US domestic adoption study. Genetic and prenatal influences moderated associations between inconsistency in overreactive parenting from child age 9 months to 4.5 years and children's cortisol variability at 4.5 years differently for mothers and fathers. Among children whose birth mothers had high morning cortisol, adoptive fathers' inconsistent overreactive parenting predicted higher cortisol variability, whereas among children with low birth mother morning cortisol adoptive fathers' inconsistent overreactive parenting predicted lower cortisol variability. Among children who experienced high levels of prenatal risk, adoptive mothers' inconsistent overreactive parenting predicted lower cortisol variability and adoptive fathers' inconsistent overreactive parenting predicted higher cortisol variability, whereas among children who experienced low levels of prenatal risk there were no associations between inconsistent overreactive parenting and children's cortisol variability. Findings supported developmental plasticity models and uncovered novel developmental, gene $\times$ environment and prenatal $\times$ environment influences on children's cortisol functioning.

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Declaration of interest

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Keywords

Adoption; cortisol; fathers; genotype-environment interaction; longitudinal; mothers; parenting

Introduction

Developmental plasticity models posit that genetic, prenatal and postnatal environmental factors influence and interact to affect children's physiological and psychological development (e.g. DelGiudice et al., 2011; Pluess & Belsky, 2011). Specific to the current inquiry, earlier influences (i.e. genetic and prenatal) are thought to influence development of the HPA axis and subsequently moderate the effects of later environmental influences (i.e. parenting, postnatal stress) on HPA axis functioning (Pluess & Belsky, 2011). Using a longitudinal adoption sample that facilitates separation of genetic and prenatal influences from later environmental influences, we examine how these factors contribute to differences in children's cortisol functioning.

Individual differences in HPA axis functioning have been examined using a variety of cortisol-based measures, including basal levels, awakening response, diurnal pattern and stress responsivity or reactivity (Gunnar & Quevedo, 2007). Day-to-day variability in diurnal cortisol may also be viewed as an indicator of how the HPA axis responds to a wide variety of day-to-day environmental changes (Almeida et al., 2009). Specifically, the extent to which cortisol is responsive to the disparate, planned and unplanned stressors that occur in daily life is considered a measure of cortisol *variability* – tapping the dynamic adjustments the HPA axis makes, increasing *and* decreasing cortisol, as an individual adapts and/or maladapts to a changing environment (DelGiudice et al., 2011). Taking this perspective, we extend existing research by investigating how biological and environmental factors contribute to differences in children's cortisol variability (see Figure 1 for conceptual model). In brief, differences in genetics (e.g. Bartels et al., 2003), prenatal environment (e.g. Gatzke-Kopp, 2010; Phillips & Jones, 2006; Pluess & Belsky, 2011) and the postnatal family environment have been shown to contribute to differences in children's day-to-day HPA function (DelGiudice et al., 2011).

Twin and candidate gene association studies have consistently demonstrated that cortisol function, particularly morning cortisol levels, is in part driven by genetic influences (see Bartels et al., 2003 for review). Prenatal influences, particularly maternal anxiety, depression and substance use during pregnancy, have been implicated in the fetal programming of HPA functioning, including diurnal cortisol patterns, variations in cortisol patterns across days (i.e. cortisol variability), and patterns of the cortisol response to stress (Dozier et al., 2006; Fisher et al., 2006; Glover et al., 2010; Jacobson et al., 1999; Zhang et al., 2005). Although each of these prenatal risks may act, at least in part, through distinct teratogenic pathways, there is evidence that the number of stressors experienced during pregnancy contributes to child development (e.g. Huizink et al., 2002). The experience of multiple prenatal stressors may exert an aggregate effect on HPA development mediated through the mothers' and fetus' HPA axis functioning and the development of the child's HPA axis (Wadhwa, 2005). Following this logic, cumulative prenatal risks (i.e. multiple stressors) may be a common pathway through which the experience of mothers' anxiety, depression and drug use together contribute to the organization of the HPA axis, potentially increasing the likelihood that the child would develop a more flexible HPA axis.

The postnatal environment also contributes to exaggerated variability in children's cortisol (e.g. Hastings et al., 2011; Hunter et al., 2011), both in terms of instability and disruptions (i.e. inconsistency) of the rearing environment (Flinn & England, 1995; Gunnar & Donzella,

2002 for review) and particularly high levels of overreactive or punitive parenting (Claessens et al., 2011; Flinn & England, 1995; see Matthews, 2002 for review). Of note, most studies examined only maternal caregiving, despite evidence of differential effects of maternal and paternal caregiving practices on child behavior (e.g. Rothbaum & Weisz, 1994) and a growing literature suggesting that fathers play a unique role in child development (e.g. Lamb, 1997). Differences have been shown in how mothers and fathers depressive symptoms influence associations between offspring's HPA function and behavioral development (e.g. Essex et al., 2011; Laurent et al., 2013). However, differences in HPA function related to maternal and paternal caregiving have not yet been examined.

Although main effects of genetic, prenatal and postnatal rearing environments have been demonstrated as important for HPA functioning, quantitative genetic theory and the present conceptual framework suggests that gene–environment and prenatal–postnatal environment interactions also play a role in the development of HPA functioning. First, there has been some support for gene $\times$ prenatal environment interactions (path a, Figure 1) in animal literature (Wadhwa, 2005). However, whether genetic influences moderate the effect of prenatal environment on children's HPA functioning has not been tested. Second, several candidate gene studies have found that specific genes interact with environmental influences, including child maltreatment, insecure attachment and stressful life events, (gene $\times$ postnatal environment interactions path b, Figure 1) to influence cortisol responsivity in infancy (e.g. Luijk et al., 2010) and adulthood (e.g. Tyrka et al., 2009). However, whether genetic influences moderate the effect of postnatal environment on HPA functioning has rarely been tested using methods other than the candidate gene approach, and rarely in samples of children. Finally, there is some evidence of prenatal $\times$ parenting environment interactions (path c; Figure 1) such that the combination of maternal substance use during pregnancy and childhood maltreatment predicted reduced cortisol reactivity (Fisher et al., 2012). Despite the hypothesized effects and these promising findings, there remains a dearth of studies investigating how interactions between genetic, prenatal and parenting influences impact children's HPA functioning. The present study addresses these gaps and test for gene–environment (paths a and b) and prenatal–postnatal environment interactions (path c) explaining differences in children's HPA functioning.

Present study

In the present study we used an adoption design to disentangle how genetic influences and both the prenatal and postnatal family environment combine to contribute to differences in children's cortisol variability. Adoption designs of children placed at birth, wherein data are collected from both birth mothers and adoptive families, provide a literal separation of the characteristics and behaviors of the birth mother (who provide genetic and prenatal influences) from the postnatal family environment provided by the adoptive parents (Leve et al., 2007). Thus, adoption designs are capable of directly testing for gene–environment and prenatal–postnatal environment interactions (Reiss & Leve, 2007; Rutter et al., 2006).

We hypothesized that: (a) higher birth mother morning cortisol levels and higher birth mother cortisol variability would exacerbate the influence of prenatal influences on children's cortisol variability, (b) higher birth mother morning cortisol levels and increased birth mother cortisol variability would exacerbate the influence of overreactive parenting on children's cortisol variability and (c) prenatal risks would exacerbate the influence of overreactive parenting on children's cortisol variability. Gender differences in cortisol level and variability were not tested because gender-specific cortisol responses are not usually found in children (Kudielka & Kirschbaum, 2005).

Method

Participants and procedures

Participants for the present study were drawn from the Early Growth and Development Study, a multisite longitudinal study of adopted children and their birth and adoptive parents (Leve et al., 2007). Data came from the first cohort consisting of 361 linked birth parents and adoptive families who were recruited via 33 adoption agencies in 10 states across USA. Recruitment criteria included the following: (1) adoption was domestic, (2) child was placed with a non-relative adoptive family, (3) child was placed prior to 3 months of age ($M = 7.11$ days postpartum, $SD = 13.28$), (4) child had no known major medical conditions and (5) the birth and adoptive parents could read or understand English at least at an eighth-grade level (see Leve et al., 2007 for further description). Sample characteristics are given in Table 1. The sample is considered representative of families participating in domestic adoptions in USA and provides a unique opportunity to separate and examine genetic and environmental effects on children's HPA functioning and behavior.

Over time, the sample participated in a series of in-person interviews. All procedures and assessments were approved by the Institutional Review Boards (IRB) of collaborating institutions. Birth mothers (BM) were interviewed (usually in their homes) at ~4 months ($n = 360$) and 4 years ($n = 318$) postpartum. Adoptive families were interviewed when their child was 9 months ($n = 358$), 18 months ($n = 353$), 27 months ($n = 340$) and 4.5 years ($n = 304$) old. For interview measures attrition was low (516%). There were 210 (48% male children) families whose BMs and children provided at least one useable saliva sample (described below) at the 4-(for BM) and 4.5-year (for adopted children) assessments (58% of the sample, see Laurent et al., 2013 for attrition information specific to the cortisol assessment). The combined pattern of missing data resulted in an analysis sample of $N = 134$ when deleted list-wise. The analysis sample differed from families who were missing data in that it included BMs with higher education attainment, AP secondary caregivers with higher education attainment, and lower levels of pregnancy complications and anxiety/depression symptoms, $\chi^2 > 4.61$, $p < 0.05$, but did not differ in ethnic representation, perinatal drug use, exposure to toxins or neonatal complications, child placement age, AP primary caregiver education, AP household income, AP or BM age at child birth, AP overreactive parenting or child internalizing or externalizing problems at 4.5 years, $\chi^2 < 3.39$, $p > 0.07$.

Measures

Cortisol—Morning and evening saliva samples were collected from BMs and the adopted children for 3 days (~4 years postpartum for birth mothers, and at 4.5 years of age for adopted children). Birth and adoptive parents were trained in sample collection (saturating salivettes before placing them in prelabeled plastic vials) in person, following identical protocols. BMs and adopted children (through their parents) were instructed to provide, on each of the 3 days, morning saliva samples at 30 min after waking and evening saliva samples at bedtime before brushing teeth. Samples were stored by participants in the refrigerator, and then mailed to the primary study site and frozen until all samples for the assessment wave were collected. Then, all samples were sent in bulk from the study site to the University of Trier Laboratory and frozen at -20°C until being used for cortisol immunoassay (DELFI procedure; see Dressendorfer et al., 1992; inter-assay coefficients of variance: 7.1–9.0%; sensitivity: 0.173 nmol/l). Samples were assayed in duplicate, with the average of the useable values taken as the level of cortisol in that sample (intra-assay coefficients of variance = 6.04% for children and 5.53% for birth mothers, see Laurent et al., 2013, for further detail). Standard procedures were used to identify and eliminate extreme outliers (e.g. samples where BMs reported a 12-h delay between waking and providing the “morning” sample were removed; cortisol values $> 2.0\mu\text{g/dl}$ and therefore biologically

improbable were removed). Next, to correct for statistical outliers and normalize data, cortisol values >2.5 SDs from the group (BM or child) and occasion (morning or evening) means were replaced with 2.5 SD values (<4% of values), log transformed, and then used to derive measures of HPA functioning. Notably, before model fitting, the use of steroid medication and illness was controlled for by regressing cortisol values on indicators of medication and illness and the residuals were used in the model below.

Of the subsample that provided saliva, over 83% of birth mothers and 88% of adopted children provided all six saliva samples. Cortisol variability could not be calculated for participants providing less than three saliva samples and so were assigned missing values for cortisol variability. About 8% of birth mothers and 6% of adopted children provided only one or two saliva samples, with the remaining ~9% of birth mothers and ~6% of adopted children providing between three and five samples. At each of the six collections, 85–93% of birth mothers and 90–95% of adopted children provided saliva.

Adoptive child cortisol variability (outcome): Children's morning cortisol was, on average, at a level of 0.60 µg/dl (SD = 0.19(µg/dl) at 12min after waking (SD = 14min) or 7:38 a.m. (SD = 43 min), and evening cortisol was, on average 0.06(µg/dl (SD = 0.03 µg/dl) at 8:12PM (SD = 51 min). Of note, "normative" levels of morning cortisol (~8:00 a.m.) in children aged 4–10 years old are often ~0.3 µg/dl (McCarthy et al., 2009). This sample was ~1 SD above those reported norms, similar to other samples of foster (Dozier et al., 2006) or adopted children (see Gunnar & Donzella, 2002).

Following the logic underlying investigations of intraindividual variability (Ram & Gerstorf, 2009), we sought to extract a measure of individuals' cortisol variability – the extent to which an individual's cortisol deviated from its usual diurnal pattern – from the repeated measures of cortisol (assessments within days within child). We operationalized cortisol variability as the "residue" cortisol circulating in the system after accounting for individual-specific diurnal declines. To do so, we fit individual-level regressions to each child's 3 days of cortisol data (up to six repeated measures), and quantified the extent of the "residual" variance unaccounted for by their typical daily cortisol trajectory (intercept and slope). Specifically, $Cortisol_{ti}$, individual i 's level of (log-transformed) cortisol at assessment t ($t = 1-6$) was modeled as

$$Cortisol_{ti} = \beta_{0i} + \beta_{1i}(time\ since\ waking_{ti}) + e_{ti}, \quad (1)$$

where the person-specific intercept, β_{0i} , represents the individual's expected level of cortisol during the post-awakening period (within 30 min of usual waking time), the person-specific slope, β_{1i} , represents the individual's usual diurnal decline with respect to time since waking, centered at the individuals' average morning collection time (i.e. average of sample collection times for samples 1, 3 and 5) and e_{ti} represents time-specific residual. The variance of this residual, $\sigma_{e_i}^2$, includes all day-specific variation in diurnal slopes and situation-specific variation cortisol responses (up to six per child) and is conceptualized as a measure of the cortisol variability. Between-person differences in these variability scores indicate (albeit not perfectly) differences in the dynamic variability in cortisol levels across the day in the same way that heart rate variability (HRV) is used as an indicator of cardiovascular health.

Birth mother HPA functioning (genetic influences): BMs' morning cortisol was, on average, at a level 0.56 µg/dl (SD = 0.24) 13 min after waking, (SD = 19 min) or 8:18 a.m. (SD = 111 min), and evening cortisol was, on average, 0.13 µg/dl (SD = 0.11) at 10:28p.m. (SD = 122min). Of note, these levels are consistent with those found in other samples of adult women (e.g. Adam & Gunnar, 2001). Using the modeling procedures described earlier, the

BM's repeated measures of cortisol were reduced to three scores, β_{0i} or *BM morning cortisol*, β_{1i} or *BM diurnal slope*, and σ_{ei}^2 or *BM cortisol variability*. As in other studies using similar sampling procedures (e.g. Adam & Gunnar, 2001), diurnal slopes were highly redundant with morning cortisol ($r = -0.90$). Given prior findings that morning cortisol shows higher heritability than evening cortisol (e.g. Bartels et al., 2003), we used only the measures of morning cortisol (β_{0i}) and cortisol variability σ_{ei}^2 in subsequent analyses.

Prenatal risk—The level of prenatal risk that a child was exposed to was quantified from BM information provided at the 4-month assessment on a pregnancy history calendar (adapted version of the life history calendar, Caspi et al., 1996) and a pregnancy screener that focused specifically on the pre- and perinatal period. Relevant to the present study, BM's answered a series of questions about their use of alcohol, cigarettes and illegal drugs during pregnancy; seven items from the Beck Depression Inventory (BDI; Beck et al., 1996) and five items from the Beck Anxiety Inventory (BAI; Beck & Steer, 1993) asking specifically about the prenatal period. Responses were scored using coding systems based on variety of risk indices (e.g. Kotelchuck, 1994; McNeil et al., 1994; Van den Bergh et al., 2005; Williams & Ross, 2007).

Anxiety and *Depression* were the sum of anxiety and depressive symptoms, respectively, that BM's reported experiencing during pregnancy. Anxiety and depressive symptoms were scored by creating quartile scores identifying the rank of anxiety or depressive symptoms. The bottom 25% of the sample were given a risk score of 1, 25–50% = 2, 50–75% = 3, 75–85% = 4 and 85–100% = 5. Approximately 30% of the BM's reached risk levels (i.e. more than moderate risk to the fetus). *Drug use* included serious use of cigarettes, alcohol, eight illegal drugs and prescription painkillers used illegally, defined in accordance with the McNeil-Sjostrom Scale which assigns weights to amounts of different drugs used; ~37% of BM's reached risk levels. Following the assignment of risk scores for each variable, we created weighted risk scores. If the risk score on a variable (i.e. anxiety, depression, cigarettes, alcohol, each drug) was 3 or greater (as per the McNeil-Sjostrom Scale), the weighted risk score = 1 (prenatal risk present), if the risk score on a variable was 2 or less, the weighted risk score = 0 (no risk). The individual risk cutoff scores for anxiety, depression and each drug were summed to create a final prenatal risk score. Approximately 52% of the BM's reached minimum risk levels of prenatal risk considering internalizing symptoms and drug use together. Sample descriptive statistics are provided in Table 2.

Postnatal overreactive parenting

Adoptive mothers' and fathers' level of overreactive parenting was measured at the 9, 18 and 27 months, and 4.5-year assessments using the over-reactivity subscale of The Parenting Scale (Arnold et al., 1993; $\alpha's > 0.75$ for mothers and fathers at each assessment). Higher scores reflected more overreactivity (displays of anger, meanness and irritability), and lower scores reflected less reactive responses to children's misbehavior.

The four repeated measures of overreactive parenting were aggregated into two derived scores: *overall overreactivity* (trait level) and *inconsistency of overreactivity* (extent of state fluctuation) for adoptive mothers and fathers, because both overall levels of harsh parenting and inconsistent parenting have been independently linked with HPA functioning in children in prior studies (e.g. Flinn & England, 1995). Specifically, overall over-reactivity was computed as the mean of a mother's or father's four repeated measures, and inconsistency was computed as the total intra-individual range (max–min) of those repeated measures. Sample descriptive statistics are given in Table 2.

Data analysis

Children's cortisol variability (i.e. the range of residual scores from Equation (1)) was regressed on BMs' morning cortisol level and cortisol variability (genetic influences), BMs' level of internalizing and drug use during pregnancy (prenatal influences), adoptive parents' overall level and inconsistency of overreactive mothering and fathering (parenting influences), as well as the hypothesized interactions covering genetic $\times$ prenatal influences, genetic $\times$ parenting influences and prenatal $\times$ parenting influences. All predictors were centered at the sample mean. Analyses were conducted using SAS, with statistical significance assessed at $p < 0.01$. Missing data were deleted list-wise, resulting in an analysis N of 134. Significant interactions and interactions approaching significance ($p < 0.05$) were probed using the Johnson-Neyman method to identify regions of significance (see Hayes & Matthes, 2009).

Results

Parameter estimates and brief notes on interpretation are given in Table 3. Overall, the predictors were significantly related to children's cortisol variability, $F(21,112) = 1.70$, $p < 0.05$, $R^2 = 0.24$. Keeping in mind the presence of interactions, there were no main effects of BM morning cortisol, BM cortisol variability or prenatal risk on child cortisol variability, $\beta_s < 0.02$, $t < 1.10$, $ps > 0.27$. Higher levels of adoptive mothers' overall overreactive parenting were associated with greater child cortisol variability at the trend level, $\beta = 0.04$, $t = 2.47$, $p < 0.05$. However, for adoptive fathers, overreactive parenting was not associated with child cortisol variability, $\beta = -0.02$, $t = -1.61$, $p = 0.11$. Adoptive parents' overreactive parenting inconsistency was not associated with cortisol variability for mothers or fathers, $\beta_s < 0.01$, $ts < 0.87$, $ps > 0.33$.

Genetic influences moderating effects of prenatal environment (a)

There was no evidence that genetic influences exacerbated the effects of prenatal risk on children's cortisol variability, as no genetic $\times$ prenatal environment interaction effects were significant, $\beta_s < 0.01$, $ts < 0.46$, $ps > 0.44$.

Genetic influences moderating effects of parenting (b)

There was some evidence that genetic influences moderated the effects of overreactive parenting, though not necessarily in the hypothesized exacerbating direction. First, BM morning cortisol moderated the effect of mothers' overreactive parenting inconsistency on children's cortisol variability at the trend-level, $\beta = -0.10$, $t = -1.92$, $p = 0.05$. Probing this interaction using Johnson-Neyman procedures revealed significant moderation for the 16% of the sample whose BM morning cortisol was $+0.82$ SDs or more above the sample mean. Among children whose BM had higher morning cortisol (i.e. the top 16% of the sample on BM morning cortisol), inconsistency in adoptive mothers' overreactive parenting was associated with lower child cortisol variability, whereas among children whose BM had lower or average morning cortisol (i.e. 84% of the sample), adoptive mothers' overreactive parenting inconsistency was not associated with children's cortisol variability.

Second, BM morning cortisol moderated the effect of adoptive fathers' overreactive parenting inconsistency on children's cortisol variability, $\beta = 0.17$, $t = 2.71$, $p < 0.01$. Probing this interaction using Johnson-Neyman procedures revealed significant moderation for the 20% of the sample whose BM morning cortisol was $+0.62$ SDs or more above the sample mean and for the 40% of the sample whose BM morning cortisol was more than -1.68 SDs below the sample mean. Among children whose BM had high morning cortisol (i.e. the top 20% of the sample for BM morning cortisol), greater inconsistency in adoptive fathers' overreactive parenting was associated with higher child cortisol variability, whereas among

children whose BM had low morning cortisol (i.e. the bottom 40% of the sample on BM morning cortisol), greater inconsistency in adoptive fathers' overreactive parenting was associated with lower child cortisol variability. Among youth whose BM had close to average morning cortisol (i.e. 40% of the sample) there was no association between fathers' overreactive parenting and child cortisol flexibility. There were no other significant interactions between BM morning cortisol and adoptive mothers' or fathers' overall overreactive parenting, or between BM cortisol flexibility and overall overreactive parenting or overreactive parenting inconsistency, $\beta_s < 0.09$, $t_s > -1.5$, $p_s > 0.14$.

Prenatal risk exposure moderating effects of parenting (c)

There was also some evidence that prenatal risk exposure moderated the effects of overreactive parenting. Prenatal risk did not moderate the effect of adoptive mothers' or fathers' overall overreactive parenting on children's cortisol variability, $\beta_s > -0.005$, $t_s > -1.32$, $p_s > 0.19$. Prenatal risk moderated the effect of mothers' overreactive parenting inconsistency on children's cortisol variability, $\beta = -0.01$, $t = -2.82$, $p < 0.01$. Probing this interaction using Johnson-Neyman procedures revealed significant moderation for the 26% of the sample whose prenatal risk was more than +1.15 SDs above the sample mean. Among children experiencing high levels of prenatal risk (i.e. the top 26% of the sample on prenatal risk exposure), a greater inconsistency in mothers' overreactive parenting was associated with lower child cortisol variability, whereas among children who experienced low or no prenatal risk (i.e. 74% of the sample), mothers' overreactive parenting inconsistency was not associated with children's cortisol variability. Prenatal risk exposure also moderated the effect of fathers' overreactive parenting inconsistency on children's cortisol flexibility, $\beta = 0.01$, $t = 3.15$, $p < 0.01$. Probing this interaction using Johnson-Neyman procedures revealed significant moderation for the 26% of the sample whose prenatal risk was +1.12 SDs or more above the sample mean. Among children experiencing high levels of prenatal risk (i.e. the top 26% of the sample on prenatal risk exposure), a greater range in fathers' overreactive parenting was associated with higher child cortisol variability, whereas among children who experienced low or no prenatal risk (i.e. 74% of the sample), fathers' overreactive parenting inconsistency was not associated with children's cortisol variability.

Discussion

The purpose of this study was to examine how biological and environmental factors contribute to differences in children's cortisol variability, and was facilitated by the use of data collected from birth mothers and adoptive families who participated in the Early Growth and Development Study. Findings suggest that repeated measures obtained from a sample of 134 children, their birth mothers, and their adoptive parents allowed separation of genetic and prenatal environment factors from family environment factors. The present study adds to the literature, showing that fluctuations in the levels of overreactive parenting behaviors children experience play an important role for children's cortisol variability in the context of genetic and prenatal risk, differently for mothers and fathers.

Results generally support developmental plasticity models for the HPA axis (e.g. Del Giudice et al., 2011) hypothesizing the role of gene-environment interactions in the development of HPA functioning. In brief, we did not find evidence that genetic influences moderated the effect of prenatal influences, but did find that genetic and prenatal environment characteristics moderated the effects of early parenting on children's cortisol variability at age 4.5 years, in somewhat unexpected ways. Generally, genetic influences (i.e. higher BM morning cortisol) and prenatal risk (i.e. maternal drug use and internalizing symptoms) moderated the influence of overreactive parenting inconsistency on children's

cortisol variability, albeit maternal and paternal parenting effects were typically in opposite directions. This pattern of results is described in more detail below.

Combined influences of genetic, prenatal and the postnatal rearing environment

Genetic and prenatal risk factors consistently moderated associations between both mothers' and fathers' overreactive parenting inconsistency and children's cortisol variability, with effects varying by parent gender. Although these effects were modest, the findings contribute to the literature on the development of HPA function by clarifying how gene–environment interplay can impact cortisol variability. Thus far, the literature has tended to focus on either genetic or environmental influences that help shape children's HPA functioning, and the extent to which children's HPA function changes as a consequence of environmental input. The present findings highlight the role of gene–environment interplay in that early organizational factors interact with later environmental cues, and that the combination of earlier (genetic and prenatal) and later (parenting) influences to produce changes in children's cortisol across days. Further, our findings highlight how earlier influences can predispose some youth but not others to be particularly influenced by the inconsistency of caregiving provided by mothers and fathers.

Differential effects of mothering versus fathering on child *behavior* have been well documented (e.g. Rothbaum & Weisz, 1994). In a recent study, maternal and paternal depressive symptoms differentially moderated associations between children's HPA function and psychopathology symptoms (Essex et al., 2011). Interestingly, the same pattern of findings for paternal depressive symptoms was found for family expressed anger, but the opposite pattern of findings was found for maternal depressive symptoms, providing evidence that paternal influences on associations between HPA function and psychopathology symptoms over time may operate on a more global level than maternal influences (Essex et al., 2011).

Our findings suggest that paternal and maternal caregiving may have different effects on HPA development as well as on behavior (Rothbaum & Weisz, 1994) and on associations between HPA functioning and behavior (Essex et al., 2011). Thus, one explanation for these findings is that differences in parenting roles and time spent parenting may differently impact children's HPA functioning and this may be a function of maternal versus paternal caregiving. Based on our findings, these different effects appear to operate only in the context of a genetic predisposition for high cortisol and prenatal cues readying the child for a stressful environment in this study. In line with the logic from Essex et al. (2011), inconsistent overreactive parenting from fathers may represent a global index of inconsistency in the family environment leading to exaggerated cortisol variability in the context of high BM morning cortisol or prenatal risk, whereas inconsistent overreactive parenting from mothers may exert a more specific influence on children's HPA functioning at this time. In sum, BM morning cortisol and prenatal influences moderated later adoptive mothers' and fathers' parenting influences on children's cortisol variability in our sample of children with elevated cortisol levels. These findings suggest that interactions among genetic and prenatal influences with the family environment are salient for the development of HPA function, and that the gene–environment interplay underlying cortisol variability may differ for mothers and fathers' parenting.

Genetic, prenatal and postnatal rearing environmental influences on children's cortisol

As noted earlier, the effects of parenting inconsistency on cortisol variability were moderated by genetic and prenatal influences. However, the effects of inconsistency in parenting were different for mothers and fathers. Inconsistency as a parenting construct is generally associated with child behavior problems (e.g. Arnold et al., 1993), though few

studies have examined the inconsistency of specific types of parenting behavior across childhood. Future research is needed to determine if these differences in mothers' and fathers' overreactive parenting are replicable.

Contrary to our expectations, there were no significant genetic $\times$ prenatal interactions. Recent evidence has suggested that prenatal risk could be a mechanism of genetic risk for child behavior problems (e.g. Maughan et al., 2004; Pemberton et al., 2010), and so an interactive model of gene–environment interplay may not be the salient mechanism for how genetic and prenatal environmental influences work together in the development of cortisol variability. Future studies should also consider whether prenatal risk serves as a mechanism of genetic influence by examining prenatal risk as a mediator of genetic influences on HPA functioning.

Limitations and future directions

There are several limitations important to consider when interpreting results. Our measurement of cortisol variability was limited to the variance present in morning and evening cortisol measures obtained over 3 days when children were 4.5 years old. More frequent assessment of cortisol (e.g. hourly) or other HPA hormones, as well as measurement at multiple ages would be preferable for tracking the development of HPA functioning during childhood and understanding how genetic, prenatal and parenting influences together impact that development. Relatedly, our measure of cortisol variability was, by necessity, a combination of measurement error (e.g. variations in actual collection times, including variability in the morning time after waking which may have been before or after the peak cortisol awakening response) and specific known, but unmeasured, factors that contribute to changes in cortisol (e.g. momentary stressors, naps, physical activity, food intake and alcohol/drug use for BMs, which were unmeasured in this study). To fully separate the action of the HPA from measurement error, these and other known factors contributing to HPA axis functioning should be assessed in future studies to help determine if the error inherent in our measure has obscured other notable associations. However, if the scores represented primarily measurement error we would not likely be able to predict much variance. The predictors explained 24% of the between-child differences in cortisol variability, suggesting that the extent of within-person variability across saliva samples unaccounted for by diurnal trends, measures a meaningful construct, systematically related to theoretically derived, hypothesized genetic, prenatal and parenting predictors (see Almeida et al., 2009).

Finally, we refer to the variation across cortisol measurements as cortisol variability, but given the design, we cannot determine how much the variation is due to changes in the child's proximal environment versus the endogenous functioning of the HPA system. Nonetheless, this is a first attempt to use cortisol variability to measure another aspect of HPA function. We suggest that future research identify and attach a substantively meaningful label for this variability (e.g. lability, flexibility, sensitivity and inconsistency, see Ram & Gerstorf, 2009 for discussion). Our findings suggest that despite the measurement limitations, parenting is particularly important for cortisol variability in combination with genetic and prenatal factors, and further that mothers' and fathers' parenting may have meaningful differences for the development of HPA function in children.

The use of an adoption design, including biological parents and adoptive mothers and fathers, was novel and allowed us to disentangle genetic, prenatal and parenting influences on cortisol variability. However, our sample was also relatively small and limited to US domestic, majority same-race adoptions. The methods and questions addressed here should

also be applied to other types of adoption samples (i.e. international, transracial) and genetically informed designs (i.e. twin studies, *in vitro* studies).

Although assessing between-family differences in overall level and inconsistency in parenting across childhood was a strength of our study, our assessment of overreactive parenting and its inconsistency made use of questionnaire-based parenting reports that were completed at unevenly and relatively widely spaced occasions rather than very regular observation or experience sampling methods (i.e. interviews, diaries, EMA). Shorter-term parenting inconsistency may operate via different mechanisms to influence children's HPA development than inconsistency over years, and should be considered in the future. Other avenues for future research include, but are not limited to, examination of specific prenatal risk factors instead of the composite risk utilized here in order to empirically determine whether there are specific pathways for different prenatal risk factors.

Conclusions

Despite these limitations, the present study makes important contributions to the literature by showing that genetic and prenatal influences moderated the effect of parenting on children's cortisol variability. The present study supports developmental plasticity models of the development of HPA functioning (e.g. Del Giudice et al., 2011; Pluess & Belsky, 2011). That is, genetic and prenatal influences may be early organizational factors that also interact with later environmental cues to influence within- and across-day changes in children's cortisol. Understanding how the factors influencing children's cortisol work together is an important initial step for prevention and intervention research utilizing HPA functioning.

It is widely known that HPA functioning is associated with behavioral outcomes, and that the associations between cortisol levels and behavior are highly context-dependent. Although we consistently showed that earlier influences facilitated associations between parenting inconsistency and children's cortisol variability through genotype $\times$ environment and prenatal $\times$ postnatal environment interaction, these mechanisms were also context dependent: mothers' and father's overreactive parenting were differentially associated with children's cortisol variability. Our interpretations of the findings are agnostic as to whether greater or lesser variability is adaptive or maladaptive. This is an empirical question that should be examined in future research. Rather, our findings provide a platform for delving into the biosocial mechanisms contributing to context-related changes in cortisol that will be important to incorporate in studies of hormone-behavior associations in the future. In conclusion, this study highlights the importance of examining contributions of both mothers' and fathers' parenting inconsistency in combination with earlier genetic and prenatal influences in order to understand the mechanisms underlying the development of HPA functioning in childhood.

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Figure 1.

Conceptual model. Main effects of genetic, prenatal environmental and parenting environmental influences (hashed lines) have been shown to influence children's HPA axis functioning. Genetic influences are hypothesized to moderate the influence of the prenatal (a) and postnatal environmental (b) influences on child cortisol variability, and prenatal environmental influences are hypothesized to moderate the influence of postnatal environmental influences (c) on child cortisol variability (solid lines).

Table 1

Sample descriptive statistics.

Variable	Birth mother	Adoptive parent 1	Adoptive parent 2
Age at child birth	24.35 (6.03)	37.4 (5.57)	38.24 (5.85)
Race			
Caucasian	70.9%	92.2%	90.9%
African–American	13.7%	3.6%	4.7%
Hispanic/Latino	6.3%	1.8%	1.6%
Multiethnic	4.1%	1.1%	1.1%
Other	5.0%	1.3%	1.7%
Median education level	High school	4-year college	4-year college
Median annual income	<\$15,000	\$125,000–	\$150,000
Employment			
Full time	35.1%	32.1%	73.9%
Part time	13.3%	18.0%	2.5%
Unemployed but looking for work	19.6%	0.4%	1.1%
Full-time homemaker	7.4%	30.5%	1.3%
Other	24.6%	19.0%	21.2%

Adoptive parent 1 was typically the mother. Sample descriptive statistics represent the first cohort of EGDS; $N = 361$.

Table 2

Descriptive statistics for study variables.

	Mean	SD	BM's morning cortisol	BM's cortisol variability	Prenatal risk	Mothers' overall OVR	Fathers' overall OVR	Mothers' OVR inconsistency	Fathers' OVR inconsistency
Genetic influences									
BM's' morning cortisol	0.56	0.19							
BM's' cortisol variability	0.16	0.09	0.20 [*]						
Prenatal influences									
Prenatal risk	3.20	4.57	-0.15	-0.07					
Overreactive parenting									
Mothers' overall OVR	2.37	0.61	0.16	0.12	-0.07				
Fathers' overall OVR	2.37	0.66	0.13	0.07	-0.11	0.33 [*]			
Mothers' OVR inconsistency	1.25	0.69	-0.08	0.10	0.04	0.28 [*]	0.08		
Fathers' OVR inconsistency	1.17	0.64	-0.005	0.10	-0.05	0.08	0.33 [*]	0.43 [*]	
Outcome									
Children's cortisol variability	0.16	0.08	-0.04	-0.01	0.15 [†]	0.16 [†]	-0.04	0.04	-0.07

SD = standard deviation; BM = birth mother; OVR = overreactive parenting. *N* = 134.^{*} *p* < 0.05;[†] *p* < 0.10.

Table 3

Genetic, prenatal and parenting influences on children's cortisol variability.

Parameter	<i>B</i>	SE	Interpretation
Intercept	0.16**	0.008	
Main effects			
BM morning cortisol	−0.03	0.03	No evidence of genetic or prenatal main effects
BM cortisol variability	−0.004	0.09	
Prenatal risk	0.002	0.002	
Mothers' overall OVR	0.04*	0.01	Some evidence of postnatal environmental main effect
Fathers' overall OVR	−0.02	0.01	
Mothers' OVR inconsistency	−0.01	0.01	
Fathers' OVR inconsistency	0.01	0.01	
Interactions			
BM morning cortisol			
X prenatal Risk	−0.01	0.01	No evidence of gene-prenatal environment interaction
X mothers' overall OVR	0.05	0.06	
X fathers' overall OVR	−0.09	0.06	Some evidence of gene-postnatal environment interaction
X mothers' OVR Inconsistency	−0.10*	0.06	
X fathers' OVR Inconsistency	0.17**	0.06	
BM cortisol variability			
X prenatal risk	0.01	0.02	No evidence of gene-prenatal environment interaction
X mothers' overall OVR	0.08	0.15	
X fathers' overall OVR	0.05	0.14	No evidence of gene-postnatal Interaction
X mothers' OVR Inconsistency	0.09	0.15	
X fathers' OVR Inconsistency	0.08	0.17	
Prenatal risk			
X mothers' overall OVR	0.0001	0.004	Some evidence of prenatal–postnatal environment interaction
X fathers' overall OVR	−0.005	0.003	
X mothers' OVR Inconsistency	−0.01**	0.003	
X fathers' OVR Inconsistency	0.01**	0.003	

Model fit: $F(21,112) = 1.70$, $p < 0.05$, $R^2 = 0.24$; $N = 134$. SE = standard error; OVR = overreactive parenting.*
 $p < 0.05$.**
 $p < 0.01$.