

# Variations in Heritability of Cortisol Reactivity to Stress as a Function of Early Familial Adversity Among 19-Month-Old Twins

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**Context:** Cortisol reactivity is a marker of vulnerability for a variety of stress-related diseases that likely arise from the contributions of both genetic and environmental sources of influence. However, little is known about gene-environment interplay in early cortisol reactivity.

**Objectives:** To examine the genetic and environmental contributions to early cortisol reactivity in a population-based sample of 19-month-old twins and to determine whether these contributions vary as a function of early familial adversity.

**Design:** A variant of the twin method, with genetic and environment contributions to cortisol reactivity estimated as a function of familial adversity. Familial adversity was defined as the presence of 7 risk factors during perinatal and postnatal development (eg, at 6 and 19 months of age): maternal smoking during pregnancy, low birth weight, low family income, low maternal educational level, single parenthood, young motherhood, and maternal hostile or reactive behaviors. Twins exposed to 4 or more risk factors at either time were considered as having been exposed to high (vs low) familial adversity (23.4% of the sample).

**Setting:** Centre de Recherche Fernand-Seguin at the Hôpital Louis-Hyppolite Lafontaine, Montréal, Quebec.

**Patients:** Participants were families of twins from the Québec Newborn Twin Study recruited between April 1, 1995, and December 31, 1998, in the greater Montréal area. A total of 346 twins, 130 monozygotic and 216 dizygotic, were included in the study.

**Main Outcome Measures:** Salivary cortisol samples were collected before and after the participating twins had been exposed to unfamiliar situations; change in cortisol over time was used as a measure of cortisol reactivity.

**Results:** Distinct patterns of genetic and environmental contributions to cortisol reactivity were evidenced as a function of familial adversity, suggesting a possible gene-environment interplay. In low-familial adversity settings that characterized most families, both genetic and unique but not shared environmental factors accounted for individual differences in cortisol reactivity, with shared genes explaining the similarity observed within twin pairs. By contrast, in conditions of high familial adversity, both shared and unique environmental factors, but not genetic factors, accounted for the variance in cortisol reactivity.

**Conclusion:** This pattern of differing genetic and environmental contributions according to familial adversity suggests that, early in life, high familial adversity may have a programming developmental effect on cortisol reactivity.

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**T**HE HYPOTHALAMIC-PITUITARY-adrenal (HPA) axis underlies both adaptive and maladaptive responses to stress.<sup>1,2</sup> Adaptive responses to stress are characterized by relatively rapid activation and inhibition of cortisol secretion,<sup>1,3</sup> a glucocorticoid hormone produced by the HPA axis. However, responses to stress may become maladaptive when they are prolonged, fail to habituate, are repeatedly activated, or are quiescent when needed.<sup>4</sup>

Large individual differences in cortisol secretion have been observed during stressful conditions.<sup>5,6</sup> Substantial deviations from normative cortisol responses to stress may result from dysfunctional HPA axis reactivity and have damaging effects over time.<sup>1,3</sup> Disrupted cortisol reactivity has been associated with behavior problems and psychiatric disorders, such as anxiety,<sup>7-9</sup> depression,<sup>10,11</sup> behavioral inhibition,<sup>12,13</sup> posttraumatic stress disorder,<sup>14,15</sup> and conduct problems.<sup>16,17</sup> Cortisol reactivity is thus a marker of vulnerability for a variety of

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stress-related diseases.<sup>2</sup> Understanding the etiology of cortisol reactivity early in development may clarify the role of early vulnerability to stress during later onset of stress-related diseases.

The interindividual variability in cortisol reactivity likely arises from the joint contributions of genetic and environmental sources of influence. However, research has mainly documented the association between adverse environments and cortisol reactivity during childhood and adolescence.<sup>18-20</sup> Cortisol reactivity has been associated with low socioeconomic status,<sup>21-23</sup> maternal depression,<sup>24,25</sup> maltreatment and abuse,<sup>26-29</sup> and exposure to community violence.<sup>30</sup>

Only a handful of studies<sup>28,31,32</sup> have documented the relationship between adverse environments, such as harsh and insensitive parenting, and cortisol reactivity during the preschool years. These studies suggest a likely contribution of early adverse environments to cortisol reactivity. However, because they remain blind to the role of genetic factors in that association, it is difficult to clearly establish the nature of this contribution.<sup>33-36</sup> Twin studies are useful to this end, but few twin studies have examined the genetic and environmental contributions to cortisol reactivity. Kirschbaum et al<sup>37</sup> showed that monozygotic twins were more similar than dizygotic twins in cortisol response to corticotropin-releasing factor injection but not with respect to physical and psychological stress. Pritchard et al<sup>38</sup> found no genetic contribution to cortisol response to overfeeding in young adult twin pairs. However, the small sample sizes (ie, fewer than 25 pairs) and the fact that these studies were conducted with young adults preclude any conclusion about possible genetic contributions to cortisol reactivity in early childhood. The genetic and environmental contributions to cortisol reactivity in early childhood have yet to be documented.

Genetic and environmental factors may contribute to early cortisol reactivity in various ways. One prevailing hypothesis states that genetic and environmental factors are likely to combine nonlinearly to affect cortisol reactivity.<sup>39</sup> Gene-environment interplay has been documented through different approaches, including variations in genetic and environmental contributions according to environmental circumstances.<sup>39,40</sup> Accordingly, 2 forms of gene-environment interplay may be anticipated from previous research. First, as suggested by a diathesis-stress model,<sup>41</sup> genetic factors could predispose a child to react with an increased cortisol response if exposed to stressful environments.<sup>42</sup> In this case, a genetic vulnerability to stress would be more likely to be expressed during adversity than with favorable conditions. Results showing higher heritability of cortisol reactivity in conditions of environmental constraints, such as high vs low familial adversity (FA), would be consistent with such a model. The findings by Caspi et al<sup>43,44</sup> that genetic liabilities (ie, low monoamine oxidase A level or serotonin transporter short allele) exacerbated the impact of adverse environments (ie, maltreatment or life stress events) on later psychopathologic conditions (ie, antisocial behavior or depression) are consistent with this form of genes  $\times$  environment process.

A second possibility is that of a developmental programming effect on the HPA axis, taking the form of a reduced genetic influence on cortisol reactivity in early

adverse conditions.<sup>45,46</sup> This idea is consistent with the well-documented increased sensitivity of stress-reactive systems to environmental influences in early development.<sup>47-50</sup> In rodents, prolonged periods of maternal separation have been associated with increased corticosterone reactivity.<sup>51-53</sup> Cross-fostering studies<sup>54,55</sup> have also shown that HPA axis reactivity is modulated by early maternal care through epigenetic processes. Confirmation that these processes operate among humans awaits, although a marked brain plasticity to environment during infancy has been documented.<sup>10,56,57</sup> In a twin design, a finding showing (1) reduced genetic contributions to cortisol reactivity among twin siblings exposed to high FA and (2) higher genetic contributions among twins exposed to low FA would be consistent with this model.

The main goals of the present study were to analyze the genetic and environmental contributions to cortisol reactivity to an unfamiliar context in 19-month-old twins and to examine variations in genetic and environmental contributions to cortisol reactivity according to early FA. At this age, children typically react with marked anxiety to unfamiliar situations and adults, but large individual differences in behavioral and physiologic responses have been documented.<sup>58-60</sup> Familial adversity was defined as a function of prenatal and postnatal risk factors previously associated with cortisol reactivity to reflect the cumulative effects of stressful environments in children's lives.<sup>40</sup>

## METHODS

### SAMPLE

This study was conducted under controlled conditions at the Centre de Recherche Fernand-Seguin at the Hôpital Louis-Hyppolite Lafontaine in Montréal, Québec. Participants were families of twins from the Québec Newborn Twin Study recruited between April 1, 1995, and December 31, 1998, in the greater Montréal area. A total of 989 families were contacted, of which 672 agreed to participate (68.0%). Twins were first seen when they were 6 months of age and then prospectively assessed for a variety of child and family characteristics. After informed consent was obtained from the parents, saliva samples were collected at 19 months (mean [SD], 18.85 [0.74] months) for 466 twins before testing, 474 twins after testing, and 423 children at both times. Interviews regarding environmental variables were conducted with the mother in 99.7% of cases.

Zygoty was determined through the Zygoty Questionnaire for Young Twins when the twins were 6 and 19 months of age,<sup>61</sup> using independent ratings of the twins' physical similarities. The DNA-based zygoty was determined for 31.3% of the same-sex twin pairs randomly selected, using 8 to 10 highly polymorphic microsatellite markers. The 2 methods yielded a concordance of 93.8%.<sup>62</sup>

### THE STRESSFUL CONTEXT: EXPOSURE TO UNFAMILIAR SITUATIONS

At 19 months of age, each participating twin was successively brought into the laboratory and exposed to 2 unfamiliar situations known to be moderately stressful at that age.<sup>59,63,64</sup> In the first situation, 1 twin and the mother were alone in a corner of a room when a woman dressed as a clown entered the room, went to the opposite corner, and invited the child to approach by offering a set of familiar toys. In the second situation, a noisy, odd-

looking, moving toy robot was placed on a platform in the opposite corner of the room. Each session lasted 280 seconds separated by 5 minutes of mother-child free play. For the first 140 seconds, the mother was asked to remain passive and not to interact with her child, responding only to child-initiated talk with brief statements (eg, "It's okay," "It's a clown"). For the last 140 seconds, the mother was allowed to do whatever she thought was needed to help her child be at ease with the stimulus.

### SALIVA COLLECTION

Salivary cortisol sampling is a noninvasive and valid way to assess HPA axis activity.<sup>65,66</sup> All saliva samples were obtained between 8:05 AM and 12:15 PM during the 19 months of laboratory visits. Mothers were instructed not to give their child anything to eat or drink 20 minutes before each sampling time. Saliva was collected before and after the unfamiliar situations (Salivette; Sarstedt, Nümbrecht, Germany) and stored at  $-80^{\circ}\text{C}$  until analysis. The posttest sample was obtained 20 minutes after the end of the procedure to capture the peak cortisol response.<sup>67</sup> All samples were analyzed in a single batch using radioimmunoassay (Diagnostic Systems Laboratories Inc, Webster, Texas). The technician was blind to the zygosity status of the samples. Intra-assay variability was less than 10%.

### ASSESSMENT OF FA

Familial adversity was assessed to reflect the putative cumulative adverse effects of environmental risk factors on children's adjustment.<sup>18</sup> In the present study, an FA index was created by combining information about 7 risk factors: maternal smoking during pregnancy, low birth weight, low family income, low maternal educational level, single parenthood, young motherhood, and maternal hostile or reactive behaviors. Information about these risk factors was collected prospectively when the twins were 6 and 19 months of age, allowing changes in some risk factors (eg, low family income) to be taken into account. A score of 1 was counted for the presence of each risk factor at each time. The scores were summed to reflect the cumulative impact of these risk factors over time, assuming equal weight for each risk factor. The FA score could thus vary from 0 to 12. A risk factor was scored if the mother smoked cigarettes across all trimesters (24.9% of the families), birth weight was lower than 2500 g (46.5%), family income was below CaD \$20 000 (19.2% and 15.4% at 6 and 19 months, respectively), the mother had not completed high school (19.0% at both 6 and 19 months), the twins were not living with both of their biological parents (5.5% and 11.0% at 6 and 19 months, respectively), and the mother was younger than 20 years when she gave birth to the twins (3.2%). Finally, a 7-item, 10-point (0 indicating not at all to 10 indicating exactly) Likert-type self-report scale was used at 6 and 19 months to assess the mother's hostile or reactive parenting toward the twins (eg, "I have shaken my baby when he or she was particularly fussy" or "I have lost my temper when he or she was particularly fussy") (Cronbach  $\alpha=0.77$  and 0.73 at 6 and 19 months, respectively).<sup>33</sup> The mother's scores for both twins were averaged, and a risk was counted if the maternal hostile or reactive behaviors were above the median.

The resulting FA index was distributed as follows: FA index of 0, 16.6%; FA index of 1, 21.7%; FA index of 2, 21.9%; FA index of 3, 16.4%; FA index of 4, 10.0%; FA index of 5, 5.1%; FA index of 6, 4.5%; FA index of 7, 1.4%; FA index of 8, 0.8%; FA index of 9, 0.5%; FA index of 10, 0.8%; and FA index of 11, 0.3%. The FA index was then dichotomized for the genetic modeling: families with an FA index of 4 or above were considered to have high levels of FA (23.4%), whereas those who scored below 4 were considered to have low levels of FA (76.6%).

**Table 1. Raw Cortisol Values and the Ratio of Change for the Total Sample According to Zygosity and Sex**

Variable	Mean (SD) Baseline Cortisol Level, $\mu\text{g/dL}$	Mean (SD) Posttest Cortisol Level, $\mu\text{g/dL}$	Mean (SD) Ratio of Change
Monozygotic twins	0.36 (0.26) (n=182)	0.35 (0.22) (n=197)	0.33 (1.08) (n=163)
Dizygotic twins	0.41 (0.33) (n=284)	0.38 (0.27) (n=277)	0.26 (0.97) (n=255)
Male twins	0.40 (0.31) (n=234)	0.37 (0.26) (n=234)	0.25 (0.96) (n=212)
Female twins	0.39 (0.30) (n=232)	0.37 (0.24) (n=240)	0.33 (1.08) (n=206)
Total sample	0.39 (0.30) (n=466)	0.37 (0.25) (n=474)	0.29 (1.07) (n=418)

SI conversion factor: To convert cortisol to nanomoles per liter, multiply by 27.588.

### STATISTICAL ANALYSIS

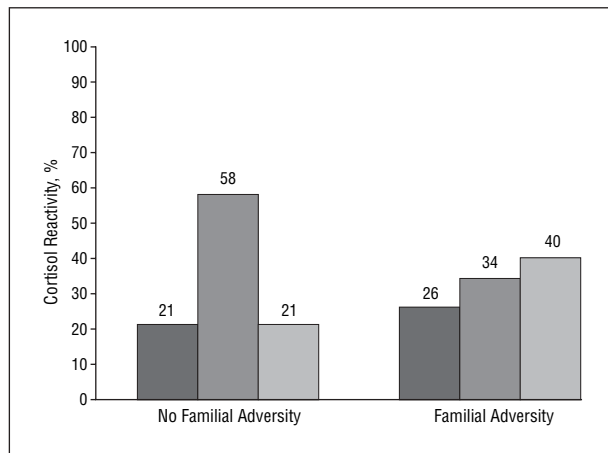
A reactive cortisol ratio was computed according to the law of initial values,<sup>68</sup> which takes into account the dependency of the posttest values on the initial values. According to the law of initial values, the change score should be adjusted if the correlation between the initial value ( $t_1$ ) and the change score ( $t_2 - t_1$  or  $\Delta t$ ) is negative, which was the case ( $r_{348} = -0.74$ ;  $P < .001$ ).<sup>67-69</sup> A cortisol reactivity ratio was thus computed by dividing  $\Delta t$  by  $t_1$ .<sup>11</sup> This score was examined for outliers, defined as values  $\pm 3$  standard deviations from the mean.<sup>67,70,71</sup> Four children (1 monozygotic and 3 dizygotic) were excluded from the subsequent analyses. Cortisol reactivity ratios were positively skewed and were normalized using a log transformation before statistical analysis.<sup>72</sup>

Differences in mean cortisol values according to zygosity and sex were investigated through analyses of variance. The association between FA and cortisol reactivity was tested using a 2-tailed  $\chi^2$  test, and multinomial logistic regressions were performed on the discrete patterns of cortisol reactivity. For the latter, children in the second and third quartiles were clustered as the reference category. All statistical tests, except for the genetic modeling, were conducted on a subsample composed of only 1 twin, selected at random, per family.

Genetic (A), shared environment (C), and unique environment (E) contributions were estimated through structural equation modeling of variance and covariance patterns among monozygotic and dizygotic pairs, using the MX software package.<sup>73</sup> All twin pairs were concordant for FA. Models that allowed parameters to vary according to FA were compared with models that constrained parameters to be equal across FA groups. Best models were selected according to goodness of fit ( $\chi^2$  test) and parsimony indices such as Akaike information criteria (AIC) and the root mean squared error of approximation (RMSEA).

### RESULTS

**Table 1** presents means and standard deviations for the pretest cortisol levels, the posttest cortisol levels, and the cortisol reactivity ratio for the total sample and as a function of zygosity and sex. The reactivity ratio did not vary according to zygosity ( $F_{1,211} = 0.37$ ;  $P = .54$ ), sex ( $F_{1,211} = 0.98$ ;  $P = .32$ ), or ethnicity (ie, white vs other;  $F_{1,158} = 0.08$ ;  $P = .59$ ). The time of day when saliva was collected was not associated with pretest or posttest cortisol levels (pretest:  $F_{1,220} = 0.37$ ;  $P = .54$ ; posttest:  $F_{1,233} = 1.06$ ;  $P = .30$ ). The FA



**Figure 1.** Distribution of the twins in the lowest, middle, and highest quartiles of cortisol reactivity according to familial adversity (n=418).

status did not differ across zygosity ( $\chi^2_{2,211}=2.45$ ;  $P=.29$ ) or sex ( $\chi^2_{2,211}=2.07$ ;  $P=.15$ ). Eleven twins were taking steroid medication occasionally. Since they did not differ significantly from the rest of the sample ( $t_{211}=0.36$ ;  $P=.55$ ) and excluding them did not affect the results, they were retained in the analyses.

No significant cortisol increase was found overall between the pretest and the posttest (mean [SD]  $\Delta t = -0.01$  [0.35]  $\mu\text{g/dL}$  [to convert to nanomoles per liter, multiply by 27.588]), except for those falling in the fourth quartile of the distribution that showed a significant cortisol increase ( $\Delta t = 0.37$  [0.26]  $\mu\text{g/dL}$ ).

#### FA AND PATTERNS OF CORTISOL REACTIVITY

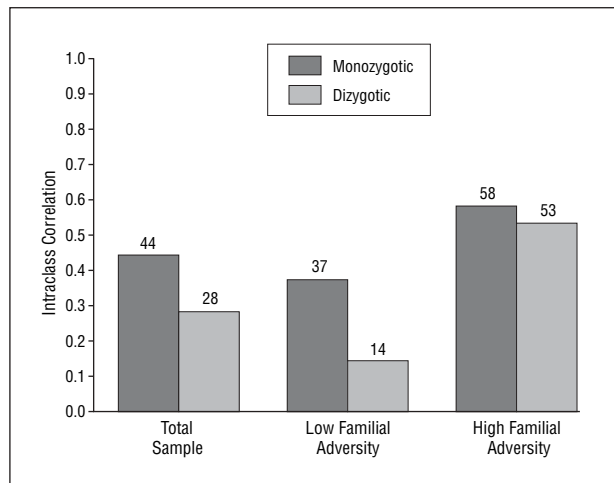
Adverse environments have been associated with both lower and higher cortisol reactivity.<sup>74</sup> We partitioned the sample into quartiles of cortisol reactivity, allowing for different associations to emerge at the lower (first quartile) and higher (fourth quartile) ends of the distribution.<sup>12,28,75</sup> **Figure 1** presents the distribution in quartiles of cortisol reactivity for high and low FA ( $\chi^2_{2,211}=9.86$ ;  $P=.007$ ).

In the low-FA group, 57.8% of twins fell into the middle quartiles, whereas 21.1% and 21.1% were assigned to the first and fourth quartiles, respectively. In the high-FA group, only 34.0% of twins were in the middle quartiles compared with 40.0% in the highest quartile and 26.0% in the lowest quartile. With the 2 middle quartiles as the reference category, multinomial logistic regression analyses revealed that high FA was linked to an increased probability of belonging to the highest quartile (Wald statistic=9.17;  $P=.007$ ) but not to the lowest quartile of the cortisol reactivity distribution (Wald statistic=3.10;  $P=.07$ ).

This pattern of association between cortisol reactivity and FA remained statistically significant when more restrictive criteria were used (ie,  $\geq 5$  risk factors) ( $\chi^2_{2,211}=11.01$ ;  $P=.004$ ) but not when more liberal criteria were used (ie,  $\geq 3$  risk factors) ( $\chi^2_{2,211}=3.97$ ;  $P=.14$ ).

#### MONOZYGOTIC-DIZYGOTIC INTRACLASSE CORRELATIONS

Only complete pairs were retained for the genetic analyses, leaving a sample of 170 pairs: 64 monozygotic pairs



**Figure 2.** Monozygotic and dizygotic intraclass correlations for cortisol reactivity according to familial adversity. Sample sizes were as follows: total: monozygotic, 64 pairs; dizygotic, 106 pairs; low familial adversity: monozygotic, 48 pairs; dizygotic, 81 pairs; high familial adversity: monozygotic, 16 pairs; dizygotic, 25 pairs.

(31 male pairs and 33 female pairs) and 106 dizygotic pairs (35 male pairs, 30 female pairs, and 41 different-sex pairs). Twins who were excluded did not differ from those selected in cortisol reactivity ( $F_{1,211}=0.15$ ;  $P=.70$ ), although they tended to experience higher FA (n=221;  $\chi^2_1=3.67$ ;  $P=.06$ ).

**Figure 2** shows the monozygotic and dizygotic intraclass correlations (ICCs) for cortisol reactivity for the total sample and according to FA. For the total sample, the ICCs were moderate to low and the monozygotic-dizygotic discrepancy was small, suggesting low heritability and little shared environment contributions overall. However, a differentiated pattern of monozygotic and dizygotic ICCs emerged according to FA. For the low-FA group, the monozygotic-dizygotic difference in correlation suggested a moderate genetic contribution, whereas for the high-FA group, both ICCs were large and of similar magnitude, suggesting a substantial shared environment but no genetic contributions (scatterplots of these monozygotic-dizygotic correlations, as well as those describing the association between pretest and posttest cortisol, are available on request). The monozygotic-dizygotic ICCs were also examined for the pretest cortisol values but did not support any of the putative models (low FA: monozygotic ICC=0.18 and dizygotic ICC=-0.11; high FA: monozygotic ICC=0.19 and dizygotic ICC=0.36).

#### GENETIC AND ENVIRONMENTAL CONTRIBUTIONS TO CORTISOL REACTIVITY

Model-fitting results for cortisol reactivity are presented in **Table 2**. We first examined models assuming the invariance of parameters across the FA groups (ie, constraining the parameters to be equal across FA groups [equal models or EQ]). Models nested within the full ACE-EQ model were fitted through the elimination of parameters. Subtracting A deteriorated the fit (model 2:  $\Delta\chi^2=4.11$ ;  $P=.04$ ), whereas removing C did not (model 3:  $\Delta\chi^2=0.00$ ;  $P>.99$ ). Removing A from model 3 resulted in worse fit ( $\Delta\chi^2=21.84$ ;  $P<.001$ ), indicating that

**Table 2. ACE Model-Fitting Results of Reactive Cortisol Level According to Familial Adversity**

Model	Fit Statistics					Estimated Components (95% CI)			
	$\chi^2$	df	P	AIC	RMSEA	Group	A	C	E
Equal FA models									
ACE	12.38	9	.19	-5.623	0.105	LFA	0.51 (0.02-0.74)	0.00 (0.00-0.34)	0.48 (0.35-0.68)
						HFA	0.51 (0.02-0.74)	0.00 (0.00-0.34)	0.48 (0.35-0.68)
CE	16.49	10	.09	-3.509	0.115	LFA		0.32 (0.17-0.50)	0.68 (0.55-0.85)
						HFA		0.32 (0.17-0.50)	0.68 (0.55-0.85)
AE	12.38	10	.26	-7.623	0.096	LFA	0.51 (0.30-0.74)		0.48 (0.35-0.68)
						HFA	0.51 (0.30-0.74)		0.48 (0.35-0.68)
E	34.22	11	<.001	12.216	0.241	LFA			1.00 (0.86-1.00)
						HFA			1.00 (0.86-1.00)
Nonequal-equal FA models <sup>a</sup>									
A <sub>1</sub> C <sub>1</sub> E <sub>1</sub> A <sub>2</sub> C <sub>2</sub> E <sub>2</sub>	5.39	6	.50	-6.612	0.044	LFA	0.40 (0.00-0.69)	0.00 (0.00-0.00)	0.60 (0.41-0.88)
						HFA	0.19 (0.00-0.82)	0.42 (0.00-0.82)	0.39 (0.20-0.72)
A <sub>1</sub> E <sub>1</sub> A <sub>2</sub> C <sub>2</sub> E <sub>2</sub>	5.39	7	.61	-8.612	0.030	LFA	0.40 (0.14-0.69)		0.60 (0.41-0.88)
						HFA	0.19 (0.00-0.82)	0.42 (0.00-0.82)	0.39 (0.20-0.72)
A <sub>1</sub> E <sub>1</sub> C <sub>2</sub> E <sub>2</sub>	5.61	8	.69	-10.392	0.025	LFA	0.40 (0.14-0.69)		0.60 (0.41-0.88)
						HFA		0.55 (0.25-0.82)	0.45 (0.30-0.73)
E <sub>1</sub> C <sub>2</sub> E <sub>2</sub>	14.26	9	.11	-3.734	0.083	LFA			1.00 (0.84-1.00)
						HFA		0.55 (0.25-0.82)	0.45 (0.30-0.73)
A <sub>1</sub> E <sub>1</sub> E <sub>2</sub>	21.10	9	.01	3.104	0.199	LFA	0.40 (0.14-0.69)		0.60 (0.41-0.88)
						HFA			1.00 (0.86-1.00)

Abbreviations: A, genetic; AIC, Akaike information criteria; C, shared environment; CI, confidence interval; E, unique environment; FA, familial adversity; HFA, high-familial adversity group; LFA, low-familial adversity group; RMSEA, root mean squared error of approximation.

<sup>a</sup>For the nonequal FA models, the parameters of the LFA are indicated as 1, whereas 2 refers to the HFA parameters.

A was essential to the fit. Consequently, the AE model was more parsimonious than the general ACE-EQ model, as confirmed by the AIC criteria. However, the RMSEA value indicated a weak fit to the data.<sup>73</sup>

We then tested models allowing the estimates in the low- and high-FA groups to differ (nonequal models or NEQ). Again, nested models were fitted through the successive elimination of parameters from the full ACE-NEQ model (model 5). Eliminating C for the low FA (model 6) or A for the high FA (model 7) did not deteriorate the fit ( $\Delta\chi^2=0.00$  and  $P>.99$  and  $\Delta\chi^2=0.22$  and  $P=.64$ , respectively), indicating that they were not useful. However, eliminating A for the low FA (model 8) or C for the high FA (model 9) weakened the fit ( $\Delta\chi^2=8.65$  and  $P=.003$  and  $\Delta\chi^2=15.49$  and  $P<.001$ , respectively). Consequently, model 7 (AE-CE) was considered the best model among the NEQ models.

In summary, models 3 and 7 were retained according to their goodness of fit ( $\chi^2$  test). Because they were not nested, these models could not be compared directly using the  $\chi^2$  test. However, based on the AIC and RMSEA values, model 7 clearly offered the best balance between explanatory power and parsimony (AIC=-10.392 and RMSEA=0.025) and was thus retained. Specifically, in low FA, cortisol reactivity was mainly accounted for by genetic (A=0.40) and unique environmental (E=0.60) factors, whereas in high FA, both shared (C=0.55) and unique (E=0.45) environmental factors contributed to cortisol reactivity. This pattern did not vary as a function of sex (data not shown).

#### COMMENT

The goals of this study were to examine the genetic and environmental contributions to cortisol reactivity in a

population-based sample of 19-month-old twins and to determine whether these contributions varied according to FA. Familial adversity was associated with higher cortisol reactivity to stress. More important, FA moderated the genetic and environmental contributions to cortisol reactivity. In low-FA settings, a condition typical of most families, both genetic and unique environmental factors accounted for individual differences in cortisol reactivity, with genes explaining the similarity observed among twin pairs. In contrast, with high FA, both shared and unique environmental factors, but not genetic factors, accounted for the variance. In the latter case, environments shared by twins in the same family explained their similarity in cortisol reactivity.

These findings are important on many accounts. First, the findings underscore the importance of FA for the expression of cortisol reactivity early in life. Previous singleton studies<sup>31,76</sup> had reported an association between disturbed patterns of cortisol reactivity and a variety of adverse environments, such as parental coercive behaviors, neglect, and poverty. However, these studies overlooked the possible gene-environment interplay in those associations.

Second, the results reveal for the first time in humans differential patterns of genetic and environmental contributions to cortisol reactivity as a function of early adverse environmental conditions. These distinct patterns are consistent with the idea that, in adverse conditions, environmental factors may have a programming developmental effect on the HPA axis that supersedes genetic factors. Stressors that provoke prolonged activation of the HPA axis early in development have been posited to inhibit ongoing neurogenesis, intensify neuronal death, and modify the localization, sensitivity, and expression of glucocorticoid receptors.<sup>10</sup> These changes could lead to long-term

alterations in HPA axis reactivity over and above genetic contributions.<sup>48,77</sup> Recent animal studies<sup>46,54</sup> have provided evidence that corticosterone reactivity in rodent offspring is mediated by maternal care, not by inherited genes. This environmental contribution has been shown to operate through an epigenetic programming effect (eg, DNA methylation) of early maternal care on the offspring's glucocorticoid receptor gene promoter expression in the hippocampus.<sup>55</sup> Whether high FA could operate through similar epigenetic mechanisms is still an open question.<sup>78</sup> High FA could also influence cortisol reactivity through its impact on immature corticolimbic structures and pathways.<sup>45</sup> Clearly, the mechanisms underlying possible early FA effects on stress reactivity among primates should be investigated further.

Third and more generally, the pattern of findings of the present study is consistent with the average expectable environment view of development.<sup>36,79</sup> According to this view, environments within the normal range are required for species-normal development but are of little value for understanding individual differences within that range. Rather, individual variations among children reared in those environments develop from genetic variation and individually experienced (ie, unique) environments, which was the case for most of our sample according to the criteria we used. The average expectable environment notion does not define the threshold of risk above which normal development may be jeopardized.<sup>80</sup> However, it is noteworthy that the range of environments covered in this sample was sufficient to reveal a different role of G and E along the continuum of FA. Similar findings suggesting low heritability of traits in the presence of social disadvantage have been reported in association with IQ<sup>81</sup> and physical health.<sup>82</sup>

Fourth, on a more practical level, the finding of a differing heritability of cortisol reactivity as a function of FA has implications for association studies aimed at identifying quantitative trait loci. The search for genetic variants underlying complex phenotypes, such as HPA axis reactivity, has revealed mixed results and lack of replication.<sup>83,84</sup> The present study suggests that early FA may confound the association between specified polymorphisms and cortisol reactivity: some polymorphisms may be relevant to cortisol reactivity for persons exposed to low FA in their childhood<sup>85</sup> although not otherwise informative for their counterparts confronted with more adverse circumstances.

Fifth, given the predictive association between cortisol reactivity and a variety of stress-related disorders (eg, depression, anxiety, and posttraumatic stress disorder),<sup>86</sup> the present findings may also have implications for clinical research and practice. They point to the relevance of planning, implementing, and assessing early preventive interventions aimed at reducing early cortisol reactivity through their mitigation of multiple FA factors in families most at risk. They also suggest that these early preventive interventions should start during pregnancy, at a time when some of the risk factors may already operate. In addition, they signal that children from families at high environmental risk, who are more likely to show high cortisol reactivity, may also benefit from most of the interventions aimed at reducing the environmental risks. Fi-

nally, they suggest that intervention models should be based on etiologic models of stress that take into account gene and environment interplay. However, because the mechanisms underlying possible early FA effects on stress reactivity are not well documented yet, they should be investigated further to more precisely establish etiologic pathways and guide preventive interventions. Early preventive trials may genuinely contribute to this endeavor by experimentally testing specific hypotheses regarding putative etiologic factors and mechanisms.

At least 5 features of the study may have constrained the findings. First, the cortisol reactivity index was based on single pretest and posttest saliva samples. In most infants, cortisol response peaks 20 minutes after stress induction, but marked individual variability exists around that point.<sup>67</sup> Multiple poststress samples may have yielded more reliable assessments of cortisol reactivity and stronger estimates of both genetic and shared-environmental contributions.

Second, for ethical reasons, a rather mild yet developmentally relevant stressful situation was used,<sup>58</sup> resulting in an absence of increase in cortisol overall. This lack of increase is consistent with previous studies<sup>87,88</sup> that used similar procedures with this age group. Unfamiliar situations do not consistently produce significant increases in cortisol for all children<sup>78</sup> but rather delineate individual differences in cortisol response to mild stressors.<sup>59,64,89,90</sup> As suggested by the large variability in the reactivity index, the unfamiliar situations were stressful for some but not for others. Many factors may have accounted for this general lack of increase in cortisol, including an already high level of cortisol before testing because of the laboratory context, the countereffect of the circadian downward trend of circulating cortisol in the morning, and the calming presence of the mother throughout the stress paradigm.<sup>78,91</sup>

Third, the present investigation of a population-based sample implied that extreme levels of FA were rare. Whether the findings generalize to the far end of the spectrum of FA is open to question. The fact that significant results were revealed despite this reduced variability in FA suggests that they are fairly robust across a considerable range of experience.

Fourth, maternal hostile or reactive behaviors relied on self-report and may have been influenced by social desirability despite the use of contextualized items (ie, the child's difficult behaviors).<sup>33,92</sup> Fifth, because FA was conceptualized as a family-level variable, it was not possible to clearly establish whether it was genuinely environmental or genetically mediated. Future studies should investigate further the nature of this gene-environment interplay by examining environmental features that are proximal to the child while showing within-family variation.<sup>39</sup>

In conclusion, the present study was the first to reveal distinct patterns of genetic and environmental contributions to early cortisol reactivity according to FA; early in life, genes may partly account for cortisol reactivity with low adversity, and adverse environments may have a programming developmental effect on cortisol reactivity. Clearly, these conditional contributions of adversity and genetic factors need to be replicated in larger samples

and further explored with respect to their timing and duration. It will also be important to determine whether they are germane to other aspects of emotion regulation. The mechanisms underlying this putative programming developmental effect of early environment should be documented to understand the early plasticity of stress-related brain structures and how they relate to later vulnerabilities and to resilience to stress and stress-related diseases, with the goal of proposing valid avenues to preventive intervention.

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## REFERENCES

- McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology*. 2000;22(2):108-124.
- McEwen BS. Protective and damaging effects of stress mediators: the good and bad sides of the response to stress. *Metabolism*. 2002;51(6)(suppl 1):2-4.
- Sapolsky RM. Is impaired neurogenesis relevant to the affective symptoms of depression? *Biol Psychiatry*. 2004;56(3):137-139.
- Lupien SJ, Ouellet-Morin I, Hupbach A, Lupien SJ, Ouellet-Morin I, Hupbach A, Tu MT, Buss C, Walker D, Pruessner J, McEwen BS. Beyond the stress concept: allostatic load—a developmental biological and cognitive perspective. In: Cicchetti D, Donald JC, eds. *Developmental Psychopathology*. Vol 2. 2nd ed. Hoboken, NJ: John Wiley & Sons; 2006:578-628.
- DeRijk RH, Schaaf M, de Kloet ER. Glucocorticoid receptor variants: clinical implications. *J Steroid Biochem Mol Biol*. 2002;81(2):103-122.
- Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*. 1994;19(4):313-333.
- Granger DA, Weisz JR, Kauneckis D. Neuroendocrine reactivity, internalizing behavior problems, and control-related cognitions in clinic-referred children and adolescents. *J Abnorm Psychol*. 1994;103(2):267-276.
- Klaassen T, Riedel WJ, van Praag HM, Menheere PP, Griez E. Neuroendocrine response to meta-chlorophenylpiperazine and ipsapirone in relation to anxiety and aggression. *Psychiatry Res*. 2002;113(1-2):29-40.
- Smider NA, Essex MJ, Kalin NH, Buss KA, Klein MH, Davidson RJ, Goldsmith HH. Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: a prospective study. *Child Dev*. 2002;73(1):75-92.
- Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. Effects of early adverse experiences on brain structure and function: clinical implications. *Biol Psychiatry*. 2000;48(8):778-790.
- Luby JL, Heffelfinger A, Mrakotsky C, Brown K, Hessler M, Spitznagel E. Alterations in stress cortisol reactivity in depressed preschoolers relative to psychiatric and no-disorder comparison groups. *Arch Gen Psychiatry*. 2003;60(12):1248-1255.
- Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. *Child Dev*. 1987;58(6):1459-1473.
- Buss KA, Schumacher JR, Dolski I, Kalin NH, Goldsmith HH, Davidson RJ. Right frontal brain activity, cortisol, and withdrawal behavior in 6-month-old infants. *Behav Neurosci*. 2003;117(1):11-20.
- Carrion VG, Weems CF, Ray RD, Glaser B, Hessel D, Reiss AL. Diurnal salivary cortisol in pediatric posttraumatic stress disorder. *Biol Psychiatry*. 2002;51(7):575-582.
- Yehuda R, Hallig SL, Grossman R. Childhood trauma and risk for PTSD: relationship to intergenerational effects of trauma, parental PTSD, and cortisol excretion. *Dev Psychopathol*. 2001;13(3):733-753.
- McBurnett K, Lahey BB, Rathouz PJ, Loeber R. Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Arch Gen Psychiatry*. 2000;57(1):38-43.
- Snoek H, Van Goozen SH, Matthys W, Buitelaar JK, van Engeland H. Stress responsiveness in children with externalizing behavior disorders. *Dev Psychopathol*. 2004;16(2):389-406.
- Evans GW. A multimethodological analysis of cumulative risk and allostatic load among rural children. *Dev Psychol*. 2003;39(5):924-933.
- Vázquez DM. Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology*. 1998;23(7):663-700.
- Heim C, Ehler U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology*. 2000;25(1):1-35.
- Evans GW, English K. The environment of poverty: multiple stressor exposure, psychophysiological stress, and socioemotional adjustment. *Child Dev*. 2002;73(4):1238-1248.
- Flinn MV, England BG. Social economics of childhood glucocorticoid stress response and health. *Am J Phys Anthropol*. 1997;102(1):33-53.
- Lupie SJ, King S, Meaney MJ, McEwen BS. Can poverty get under your skin? basal cortisol levels and cognitive function in children from low and high socioeconomic status. *Dev Psychopathol*. 2001;13(3):653-676.
- Ashman SB, Dawson G, Panagiotides H, Yamada E, Wilkinson CW. Stress hormone levels of children of depressed mothers. *Dev Psychopathol*. 2002;14(2):333-349.
- Lupien SJ, King S, Meaney MJ, McEwen BS. Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biol Psychiatry*. 2000;48(10):976-980.
- Cicchetti D, Rogosch FA. Diverse patterns of neuroendocrine activity in maltreated children. *Dev Psychopathol*. 2001;13(3):677-693.
- Glaser D. Child abuse and neglect and the brain: a review. *J Child Psychol Psychiatry*. 2000;41(1):97-116.
- Hart J, Gunnar M, Cicchetti D. Salivary cortisol in maltreated children: evidence of relations between neuroendocrine activity and social competence. *Dev Psychopathol*. 1995;7(1):11-26.
- Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH, Nemeroff CB. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA*. 2000;284(5):592-597.
- Scarpa A, Ollendick TH. Community violence exposure in a young adult sample, III: psychophysiology and victimization interact to affect risk for aggression. *J Community Psychol*. 2003;31(4):321-338.
- Bugental DB, Martorell GA, Barraza V. The hormonal costs of subtle forms of infant maltreatment. *Horm Behav*. 2003;43(1):237-244.
- Gunnar MR, Morison SJ, Chisholm K, Schuder M. Salivary cortisol levels in children adopted from Romanian orphanages. *Dev Psychopathol*. 2001;13(3):611-628.
- Boivin M, Perusse D, Dionne G, Sayset V, Zoccolillo M, Tarabulsy GM, Tremblay N, Tremblay RE. The genetic-environmental etiology of parents' percep-

- tions and self-assessed behaviours toward their 5-month-old infants in a large twin and singleton sample. *J Child Psychol Psychiatry*. 2005;46(6):612-630.
34. Dionne G, Dale PS, Boivin M, Plomin R. Genetic evidence for bidirectional effects of early lexical and grammatical development. *Child Dev*. 2003;74(2):394-412.
  35. Plomin R, DeFries JC, McClearn GE, Rutter M. *Behavioral Genetics*. New York, NY: Worth Publishers; 2001.
  36. Scarr S. Developmental theories for the 1990s: development and individual differences. *Child Dev*. 1992;63(1):1-19.
  37. Kirschbaum C, Wüst S, Faig HG, Hellhammer DH. Heritability of cortisol responses to human corticotropin-releasing hormone, ergometry, and psychological stress in human. *J Clin Endocrinol Metab*. 1992;75(6):1526-1530.
  38. Pritchard J, Després JP, Gagnon J, Tchernof A, Nadeau A, Tremblay A, Bouchard C. Plasma adrenal, gonadal, and conjugated steroids before and after long term overfeeding in identical twins. *J Clin Endocrinol Metab*. 1998;83(9):3277-3284.
  39. Rutter M, Moffitt TE, Caspi A. Gene-environment interplay and psychopathology: multiple varieties but real effects. *J Child Psychol Psychiatry*. 2006;47(3-4):226-261.
  40. Moffitt TE, Caspi A, Rutter M. Strategy for investigating interactions between measured genes and measured environments. *Arch Gen Psychiatry*. 2005;62(5):473-481.
  41. Zubin J, Spring B. Vulnerability: a new view of schizophrenia. *J Abnorm Psychol*. 1977;86(2):103-126.
  42. McEwen BS, Lasley EN. *The End of Stress as We Know It*. Washington, DC: John Henry Press; 2002.
  43. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R. Role of genotype in the cycle of violence in maltreated children. *Science*. 2002;297(5582):851-844.
  44. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003;301(5631):386-389.
  45. Gunnar M, Vazquez DM. Stress neurobiology and developmental psychopathology. In: Cicchetti D, Donald JC, eds. *Developmental Psychopathology*. Vol 2. Hoboken, NJ: John Wiley & Sons; 2006:533-577.
  46. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci*. 2001;24:1161-1192.
  47. Casey BJ. Brain, plasticity, learning, and developmental disabilities. *Ment Retard Dev Disabil Res Rev*. 2003;9(3):133-134.
  48. De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocr Rev*. 1998;19(3):269-301.
  49. Hardie TL, Moss HB, Vanyukov MM, Yao JK, Kirilovac GP. Does adverse family environment or sex matter in the salivary cortisol responses to anticipatory stress? *Psychiatry Res*. 2002;112(2):121-131.
  50. Schulkin J. Allostasis: a neural behavioral perspective. *Horm Behav*. 2003;43(1):21-27.
  51. Plotsky PM, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res Mol Brain Res*. 1993;18(3):195-200.
  52. Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring. *J Neurosci*. 1997;17(7):2626-2636.
  53. Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. The effects of early life events on in vivo release of norepinephrine in the paraventricular nucleus of the hypothalamus and hypothalamic-pituitary-adrenal responses during stress. *J Neuroendocrinol*. 2000;12(1):5-12.
  54. Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*. 1999;286(5442):1155-1158.
  55. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004;7(8):847-854.
  56. Gunnar MR, Donzella B. Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*. 2002;27(1-2):199-220.
  57. Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM. The neurobiological consequences of early stress and childhood maltreatment. *Neurosci Biobehav Rev*. 2003;27(1-2):33-44.
  58. Buss KA, Davidson RJ, Kalin NH, Goldsmith HH. Context-specific freezing and associated physiological reactivity as a dysregulated fear response. *Dev Psychol*. 2004;40(4):583-594.
  59. van Bakel HJ, Riksen-Walraven JM. Stress reactivity in 15-month-old infants. *Dev Psychobiol*. 2004;44(3):157-167.
  60. Kagan J. Biology and the child. In: Eisenberg N, ed. *Social, Emotional, and Personality Development*. Vol 3. 5th ed. New York, NY: Wiley; 1998:177-235.
  61. Goldsmith HH. A zygosity questionnaire for young twins: a research note. *Behav Genet*. 1991;21(3):257-269.
  62. Forget-Dubois N, Perusse D, Turecki G, Girard A, Billette JM, Rouleau G, Boivin M, Malo J, Tremblay RE. Diagnosing zygosity in infant twins: physical similarity, genotyping, and chorionicity. *Twin Res*. 2003;6(6):479-485.
  63. Mullen M, Snidman N, Kagan J. Free-play behavior in inhibited and uninhibited children. *Infant Behav Dev*. 1993;16:383-389.
  64. Goldberg S, Levitan R, Leung E, Masellis M, Basile VS, Nemeroff CB, Atkinson L. Cortisol concentrations in 12- to 18-month-old infants: stability over time, location, and stressor. *Biol Psychiatry*. 2003;54(7):719-726.
  65. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology*. 1989;22(3):150-169.
  66. Schwartz EB, Granger DA, Susman EJ, Gunnar MR, Laird B. Assessing salivary cortisol in studies of child development. *Child Dev*. 1998;69(6):1503-1513.
  67. Ramsay D, Lewis M. Reactivity and regulation in cortisol and behavioral responses to stress. *Child Dev*. 2003;74(2):456-464.
  68. Lacey JI. The evaluation of autonomic responses: toward a general solution. *Ann N Y Acad Sci*. 1956;67:123-163.
  69. Lewis M, Ramsay DS. Effect of maternal soothing on infant stress response. *Child Dev*. 1999;70(1):11-20.
  70. Gunnar MR, Connors J, Isensee J. Lack of stability in neonatal adrenocortical reactivity because of rapid habituation of the adrenocortical response. *Dev Psychobiol*. 1989;22(3):221-233.
  71. Grunau RE, Weinberg J, Whitfield MF. Neonatal procedural pain and preterm infant cortisol response to novelty at 8 months. *Pediatrics*. 2004;114(1):e77-e84.
  72. Tabachnick BG, Fidell LS. *Using Multivariate Statistics*. 3rd ed. New York, NY: HarperCollins College Publishers; 1996.
  73. Neale MC, Boker SM, Xie G, Maes HH. *MX: Statistical Modeling*. 5th ed. Richmond: Department of Psychiatry, Medical College of Virginia Commonwealth University; 1999.
  74. Charmandari E, Kino T, Souvatzoglou E, Chrousos GP. Pediatric stress: hormonal mediators and human development. *Horm Res*. 2003;59(4):161-179.
  75. Ellis BJ, Essex MJ, Boyce TW. Biological sensitivity to context, II: empirical explorations of an evolutionary-developmental theory. *Dev Psychopathol*. 2005;17(2):303-328.
  76. Gunnar MR, Brodersen L, Nachmias M, Buss K, Rigatuso J. Stress reactivity and attachment security. *Dev Psychobiol*. 1996;29(3):191-204.
  77. Repetti RL, Taylor SE, Seeman TE. Risky families: family social environments and the mental and physical health of offspring. *Psychol Bull*. 2002;128(2):330-366.
  78. Gunnar M, Quevedo K. The neurobiology of stress and development. *Annu Rev Psychol*. 2007;58:145-173.
  79. Scarr S. Biological and cultural diversity: the legacy of Darwin for development. *Child Dev*. 1993;64(5):1333-1353.
  80. Baumrind D. The average expectable environment is not good enough: a response to Scarr. *Child Dev*. 1993;64(5):1299-1317.
  81. Turkheimer E, Haley A, Waldron M, D'Onofrio B, Gottesman II. Socioeconomic status modifies heritability of IQ in young children. *Psychol Sci*. 2003;14(6):623-628.
  82. Johnson W, Krueger RF. Genetic effects on physical health: lower at higher income levels. *Behav Genet*. 2005;35(5):579-590.
  83. Rosmond R, Chagnon YC, Chagnon M, Perusse L, Bouchard C, Bjorntorp P. A polymorphism of the 5'-flanking region of the glucocorticoid receptor gene locus is associated with basal cortisol secretion in men. *Metabolism*. 2000;49(9):1197-1199.
  84. Wüst S, Van Rossum EF, Federenko IS, Koper JW, Kumsta R, Hellhammer DH. Common polymorphisms in the glucocorticoid receptor gene are associated with adrenocortical responses to psychosocial stress. *J Clin Endocrinol Metab*. 2004;89(2):565-573.
  85. Lakatos K, Nemoda Z, Birkas E, Ronai Z, Kovacs E, Ney K, Toth I, Sasvari-Szekely M, Gervai J. Association of D4 dopamine receptor gene and serotonin transporter promoter polymorphisms with infants' response to novelty. *Mol Psychiatry*. 2003;8(1):90-97.
  86. Pollak SD. Early adversity and mechanisms of plasticity. *Dev Psychopathol*. 2005;17(3):735-752.
  87. Gunnar MR, Larson MC, Hertzgaard L, Harris ML, Brodersen L. The stressfulness of separation among nine-month-old infants: effects of social context variables and infant temperament. *Child Dev*. 1992;63(2):290-303.
  88. Hertzgaard L, Gunnar M, Erickson MF, Nachmias M. Adrenocortical responses to the strange situation in infants with disorganized/disoriented attachment relationships. *Child Dev*. 1995;66(4):1100-1106.
  89. Garcia Coll C, Kagan J, Reznick JS. Behavioral inhibition in young children. *Child Dev*. 1984;55:1005-1019.
  90. Parritz RH. A descriptive analysis of toddler coping in challenging circumstances. *Infant Behav Dev*. 1996;19:171-180.
  91. Gunnar MR, Brodersen L, Krueger K, Rigatuso J. Dampening of adrenocortical responses during infancy. *Child Dev*. 1996;67(3):877-889.
  92. Morsbach SK, Prinz RJ. Understanding and improving the validity of self-report of parenting. *Clin Child Fam Psychol Rev*. 2006;9(1):1-21.



- Area surveys: the control and measurement of error. *Arch Gen Psychiatry*. 1984; 41(10):942-948.
40. Eaton WW, Kalaydjian A, Scharfstein DO, Mezuk BM, Ding Y. Prevalence and incidence of depressive disorder: the Baltimore ECA followup, 1981-2004. *Acta Psychiatr Scand*. 2007;116(3):182-188.
  41. Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, Rush AJ, Weissman MM. Conceptualization and rationale for consensus definition of terms in major depressive disorder. *Arch Gen Psychiatry*. 1991;48(9):851-855.
  42. Mezuk BM, Zandi P, Eaton WW. Participant characteristics that influence consent for genetic research in a population-based survey: the Baltimore Epidemiologic Catchment Area Followup. *Community Genet*. In press.
  43. Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES, Easton DF, Rubinsztein DC. Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. *Am J Med Genet*. 1998; 81(1):58-63.
  44. Lawless JF. *Statistical Models and Methods for Lifetime Data*. New York: John Wiley & Sons; 1982.
  45. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika*. 1986;73(1):13-22.
  46. Eaton WW, Neufeld K, Chen L, Cai G. A comparison of self-report and clinical diagnostic interviews for depression: DIS and SCAN in the Baltimore ECA Followup. *Arch Gen Psychiatry*. 2000;57(3):217-222.
  47. Prien RF, Carpenter LL, Kupfer DJ. The definition and operational criteria for treatment outcome of major depressive disorder: a review of the current research literature. *Arch Gen Psychiatry*. 1991;48(9):796-800.
  48. Van Londen L, Molenaar RP, Goekoop JG, Zwinderman AH, Rooijmans HG. Three- to 5-year prospective follow-up of outcome in major depression. *Psychol Med*. 1998;28(3):731-735.
  49. Judd LL, Akiskal HS, Maser JD, Zeller PJ, Endicott J, Coryell W, Paulus MP, Kunovac JL, Leon AC, Mueller TI, Rice JA, Keller MB. A prospective 12-year study of subsyndromal and syndromal depressive symptoms in unipolar major depressive disorders. *Arch Gen Psychiatry*. 1998;55(8):694-700.
  50. Angst J, Merikangas K. The depressive spectrum: diagnostic classification and course. *J Affect Disord*. 1997;45(1-2):31-40.
  51. Eaton WW. Studying the natural history of psychopathology. In: Tsuang MT, Tohen M, eds. *Textbook in Psychiatric Epidemiology*. 2nd ed. New York: Wiley-Liss; 2002:215-238.
  52. Simpson HB, Nee JC, Endicott J. First-episode major depression: few sex differences in course. *Arch Gen Psychiatry*. 1997;54(7):633-639.

### Correction

**Errors in Byline and Author Affiliations.** In the Original Article titled "Variations in Heritability of Cortisol Reactivity to Stress as a Function of Early Familial Adversity Among 19-Month-Old Twins" by Ouellet-Morin et al, published in the February issue of the *Archives* (2008;65[2]:211-218), errors occurred in the byline on page 211 and in the Author Affiliations on page 217. In the byline, the fifth author's name should have been listed as "Louise Arseneault, PhD." On page 217, in the Author Affiliations, the second-to-last affiliation should have appeared as follows: "Institute of Psychiatry, King's College, London, England (Dr Arseneault)."