Daytime Cortisol Secretion in 6-Month-Old Twins: Genetic and Environmental Contributions as a Function of Early Familial Adversity

Isabelle Ouellet-Morin, Ginette Dionne, Daniel Pérusse, Sonia J. Lupien, Louise Arseneault, Ronald G. Barr, Richard E. Tremblay, and Michel Boivin

Background: Dysregulation of daytime cortisol activity has been associated with stress-related pathologies. Research suggests that early environmental adversity might shape cortisol activity. However, little is known about the genetic and environmental contributions to early cortisol and how this varies as a function of environmental circumstances. The goals of the study were to estimate the genetic and environmental contributions to daytime cortisol secretion in infant twins and to investigate whether these contributions varied as a function of familial adversity (FA).

Methods: Participants were 517 6-month-old twins. Salivary cortisol was collected when the infants woke up at home and in the morning, upon arrival at the laboratory. Familial adversity was defined by seven perinatal and postnatal risk factors: maternal smoking during pregnancy, low birth weight, low family income, low maternal education, single parenthood, young motherhood, and maternal hostile/reactive behaviors. Genetic and environment contributions to cortisol activity were estimated for high (three risk factors or more: 21.3% of the sample) versus low FA.

Results: Genetic factors accounted for cortisol levels in different ways: a moderate "main effect" of genes was found for home-based awakening cortisol, whereas the contribution of genes to morning cortisol was conditional to FA. Genetic factors accounted for most of the variance in morning cortisol in high family adversity but not in low family adversity.

Conclusions: Early FA modulates the heritability of morning cortisol in infants. The results are consistent with the diathesis-stress model, with genetic factors more likely to be expressed in adverse settings.

Key Words: Cortisol, early adversity, genetic–environment interaction (G×E), HPA axis, stress, twin study

The Hypothalamus-Pituitary-Adrenal (HPA) axis activity underlies the organism's response to stressful conditions (1). Cortisol, the end-product of the HPA axis, peaks shortly after awakening and progressively decreases throughout the day. This circadian cycle is established within the first months of life (2–4). Whereas cortisol generally helps the organism face daily life obligations, disturbed patterns of cortisol secretion are potentially detrimental in the long run (1). Dysregulation of daytime cortisol activity has indeed been associated with stress-related pathologies, including depression (5,6), post-traumatic stress disorder (7,8), anxiety (9), externalizing behaviors (10), obesity (11), and cognitive deficits (12,13). Describing the causes of early cortisol secretion is thus an important step in understanding the vulnerability to later stress-related diseases.

Research has documented an association between disrupted daytime cortisol and markers of adversity, such as low familial socioeconomic status (14), economic poverty (15), single motherhood (16), low birth weight (17), prenatal alcohol and cigarette exposure (18), neglect (19,20), and abuses (21–24). These findings suggest that early adversity might shape cortisol activity (25–30). However, because these studies did not consider the role of genetic factors in predicting cortisol, the role of environmental adversity is still open to debate (31–33).

Individual differences in daytime cortisol levels likely arise from the joint contribution of genetic and environmental factors. A handful of twin studies have examined the genetic–environmental etiology of daytime cortisol activity. They suggest a substantial heritability and no shared environment contribution to daytime cortisol (34) (35–39). However, most of these studies relied on older and age-heterogeneous samples of twins. Due to the plasticity of the yet immature but fast-developing brain structures during the first years of life, early environmental adversity could influence cortisol activity in decisive ways (40,41). Accordingly, the present study focused on the gene environment processes underlying daytime cortisol in infancy.

The genetic and environmental contributions to daytime cortisol vary as a function of the time of the day: moderate to high heritability has been reported at awakening in adults but not later during the day (42). The only gene environment study of daytime cortisol involving an age-homogenous sample of children (12-year-old) revealed a genetic contribution in the morning and early afternoon but not in the evening (34), with the morning samples showing the highest heritability. Thus, there seems to be a gradual circadian shift from genetic to environmental control. However, this environmental contribution does not seem to be experienced similarly by children of the same family (34,43), which points to possible genetic and environmental (G × E) interactions.

Gene–environment interplay has been reported for various health-related phenotypes and with different approaches (44–

From the School of Psychology (IO-M, GD, MB), Université Laval, Québec City; Department of Anthropology (DP), Université de Montréal; Center for Human Stress Research (SJL), Douglas Hospital Research Center, McGill University, Montréal; Child and Family Research Institute (RGB), University of British Columbia, Vancouver, Canada; Institute of Psychiatry (LA), King's College, London, United Kingdom; and the Department of Psychology (RET), Université de Montréal, Montréal, Canada, and International Laboratory for Child and Adolescent Mental Health Development, INSERM U669, Paris, France.

Address reprint requests to Michel Boivin, Ph.D., École de Psychologie, 2325 rue des Bibliothèques, Pavillon Félix-Antoine-Savard, Université Laval, Québec, Canada, G1V 0A6; E-mail: michel.boivin@psy.ulaval.ca.

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47), including variations in genetic and environmental etiology according to environmental circumstances (48,49). At least two forms of gene-environment interplay might be anticipated. A first possibility, often defined as the "diathesis-stress" model (50), posits that genes that increase vulnerability (or resilience) to stress are more likely to be expressed under adverse/stressful environments than under more favorable conditions (29,51–54). Results showing higher heritability of daytime cortisol secretion under stressful conditions, such as high versus low familial adversity (FA), would be consistent with such a model.

Early adverse environments might also constrain genetic expression. In rodents, early maternal care has long-lasting effect on the HPA axis response to stress (55,56). Findings showing a reduced genetic contribution to cortisol among infants exposed to stressful conditions, such as high versus low FA, would be consistent with that model.

The goal of the present study was to estimate the genetic and environmental contributions to morning cortisol secretion among 6-month-old twins and to investigate whether and how these contributions varied as a function of FA.

Methods and Materials

Participants

Participants were twins recruited between April 1995 and December 1998 in the greater Montréal area to participate in a longitudinal study. A total of 989 families were contacted, of which 672 agreed to participate (68%). Twins were first seen when they were 6 months of gestational age and then prospectively assessed on a variety of child and family characteristics. Informed consent was obtained from the parents annually. Interviews regarding environmental variables were generally conducted with the mother (99.7%). Hospital records were used to get information about pregnancy and delivery. Zygosity was determined through the Zygosity Questionnaire for Young Twins when they were 6 and 19 months of age (57). The DNA-based zygosity was determined for 31% of randomly selected same-sex twin pairs with 8–10 highly polymorphic micro-satellite markers. The two methods yielded a concordance of 93.8% (58).

Saliva samples were collected for 523 children when they were 6 months of age (mean [SD], 5.63 months [.93]). Three twin pairs were excluded, because they were born very premature (26–29 gestational weeks) and with a very low birth weight (≤1000 g), two conditions associated with disturbed HPA axis activity (59,60). The final sample was composed of 517 infants who participated in cortisol sampling at least once (n = 478 and n = 393 for awakening and morning, respectively). Non-genetic statistical analyses (e.g., analyses of variance) were performed with all available twins, but only complete twin pairs were considered for genetic analyses, leaving 232 twin pairs (101 monozygotic [MZ] and 131 dizygotic [DZ] pairs) and 192 twin pairs (67 MZ and 125 DZ pairs) for the awakening and morning samples, respectively. Cortisol levels of infants from complete pairs did not differ from cortisol levels of singleton twins [$t(248) = -1.62$, $p = .11$, and $t(201) = -1.45$, $p = .15$].

Procedures and Measures

Saliva Collection. Two saliva samples were collected 1 week apart: 1) "home-awakening" and 2) "lab in the morning." The "lab in the morning" samples were collected first, immediately upon arrival at the laboratory (approximately 15 min, between 8:32 AM and 10:02 AM; mean [SD], 8:55 [0:14]) with salivettes (Sarstedt Canada, St-Laurent, Québec). Parents were instructed to collect the saliva at home 7 days later, as soon as the child naturally awakes and when he or she is still lying in the bed, unfed. Families were reminded by phone to do so the day before the sampling. Parents were told to put the salivettes in their freezer until the home visit scheduled the following week. The salivettes were brought back to the laboratory and stored at −80°C until assay. The "home-awakening" samples were all collected between 6:00 AM and 10:00 AM (mean [SD], 7:29 AM [0:56]). Mothers were instructed not to feed or give the child anything to drink 20 min before each sampling. All samples were analyzed in a single batch with RadioImmunoAssay (Diagnostic Systems Laboratories, Webster, Texas). The technician was blind as to the zygosity and FA status of the samples. Intra-assay variability was < 10%. Cortisol levels were positively skewed and were normalized with a Log10 transformation (61).

FA

The cumulated risk of FA was assessed by seven perinatal and postnatal risk factors (27): maternal smoking during pregnancy, low birth weight, low family income, low maternal education, single parenthood, young motherhood, and maternal hostile-reactive behaviors. 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 CDN $20,000 (19.2%), the mother had not completed high school (19.0%), the twins were not living with both biological parents (5.5%), and the mother was younger than 20 years when the twins were born (3.2%). A seven-item, 10-point-Likert (0 = "not at all" to 10 = "exactly") self-report scale was used to assess the mother's hostile-reactive parenting toward each twin (e.g., "I have shaken my baby when he/she was particularly fussy") (Cronbach α = .73) (31). The mother's hostile-reactive scores were strongly correlated across twins of the same family (r_{MZ} = .84, p = .00; r_{MZ} = .78, p = .00) and were thus averaged within families. A risk was counted if the score was above the median.

The resulting FA index was distributed as follows: an FA of 0: 19.2%; 1: 30.5%; 2: 29.0%; 3: 15.0%; 4: 3.5%; 5: 1.6%; 6: 1.2%; and no FA of 7. Families with an FA score of 3 or above were considered to have high levels of FA (21.3%), and those who scored below 3 were considered to have low levels of FA (78.7%). This partition identified an FA risk group that was prevalent enough to conduct meaningful statistical analyses.

Data Analyses

Because having two twins per family implies non-independent observations, differences between groups were tested with two-level hierarchical mixed models (62), with both fixed and random components allowing an unbiased test of difference in means (63,64). Phenotypic associations were tested with intraclass correlations, controlling for zygosity (65), through MPlus (66).

Genetic Modeling

The twin design compares the phenotypic similarity of MZ twins of the same family (100% genetically related) with that of DZ twins of the same family (approximately 50% genetically related) and decomposes the phenotypic variance into three components: additive genetic variance (heritability), shared (common) environmental variance, and non-shared (unique) environmental variance (67). Genetic sources of variance are implied when MZ twins are more similar than DZ twins. Shared environment is indicated when both MZ and DZ pairs are significantly similar; it refers to environmental factors that make twins of the same family similar to each other (e.g., socioeconomic