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Heritability of Cortisol Regulation in Children

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Background: The normal development of cortisol regulation during childhood is thought to be influenced by a complex interplay between environmental and genetic factors. *Method:* The aim of this study was to estimate genetic and environmental influences on basal cortisol levels in a sample of 151 twin pairs aged 9–16 years. Salivary cortisol was collected on two consecutive days when the children attended school — immediately after awakening, 30 min post-awakening and at bedtime. *Results:* Heritability was highest (60%) for cortisol levels about 30 min after awakening. For samples taken immediately at awakening heritability was less pronounced (28%) and in the evening low (8%). *Conclusion:* The limited genetic influence on evening levels, moderate on cortisol at awakening and high on awakening response, might imply two genetic regulation patterns, one specifically for awakening response and one for the circadian rhythm proper. These findings could explain divergent results in previous studies and highlight the importance of taking the circadian rhythm into account in studies of cortisol levels in children.

■ **Keywords:** child, twin study, heritability, basal cortisol, circadian rhythm, hypothalamic–pituitary–adrenal (HPA) axis

The hypothalamic–pituitary–adrenal (HPA) axis, with cortisol as the main end product, mediates the physiological stress response by a multitude of metabolic, immunological and central nervous effects (Sapolsky et al., 2000; Sorrells & Sapolsky, 2007). In addition to acute increases in cortisol concentrations in response to threatening exposures, cortisol levels increase rapidly upon awakening — a phenomenon described as the cortisol awakening response (CAR, Fries et al., 2009; Pruessner et al., 1997). Subsequently, the cortisol secretion follows a circadian rhythm, with decreasing levels during the day, reaching low levels in the evening, with a nadir at night and slowly rising levels in the early morning before the CAR is elicited again upon awakening. Although results are inconsistent (e.g., Young & Sweeting, 2011, Jefferies et al., 2003), several studies have reported that individual differences in cortisol levels and circadian patterns within the normal range are associated with both physical (Dekker et al., 2008; Matthews et al., 2006) and mental health problems (Delahanty et al., 2005; Goodyer et al., 2001; King et al., 1998; McBurnett et al., 2000). Given these potential health-related influences of the basal cortisol regulation, investigations of its genetic and environmental determinants in childhood is of importance.

The normal development of cortisol regulation during childhood is thought to be influenced by complex interplay between environmental and genetic factors (Gunnar & Quevedo, 2007). A wide range of psychosocial circumstances have been linked to a hyperactive HPA axis in children (Gustafsson et al., 2006; Lupien et al., 2000; King et al., 2000; Pfeffer et al., 2007), and the endocrinological impact may be moderated by common genetic variants (DeRijk et al., 2006). HPA dysregulations may be present decades after hazardous exposures in childhood (Gustafsson et al., 2010b); an observation that further

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emphasizes the importance of studying the HPA functioning in young subjects. In a meta-analysis of five twin studies examining the heritability of basal cortisol levels (Bartels et al., 2003b), the genetic component for basal cortisol levels was estimated to be 62%. More recent studies indicate that the heritability of cortisol levels may vary for different parts of the circadian rhythm. Specifically, one study of 12-year-old twin pairs revealed a high heritability for cortisol levels 45 min after awakening, but lower heritability for evening levels (Bartels et al., 2003a). The finding of a substantial genetic influence on cortisol levels in the early morning, but not in the evening, has been generally supported by more recent twin studies, which have found low heritability for evening levels in preadolescent children (Schreiber et al., 2006) and a more pronounced genetic influence on cortisol levels around awakening than on mid-morning levels in infants (Ouellet-Morin et al., 2009). Kupper and co-workers (Kupper et al., 2005) reported a high heritability for cortisol levels in proximity to awakening, but not during the later part of the day. Contrasting results have, however, been reported in one study of preadolescent children (Steptoe et al., 2009), reporting a high heritability (58%) of basal cortisol levels in the afternoon (morning cortisol not sampled).

To the best of our knowledge, only one prior twin study has assessed absolute cortisol levels in the morning separately from the CAR (Wust et al., 2000a). The CAR has been given increased attention during the last decade (Chida & Steptoe, 2009; Fries et al., 2009) as a conveniently measured part of the circadian rhythm. Prior evidence support that normal functioning of the hippocampus, exerting primarily inhibitory influences on the HPA axis (Herman et al., 2003), is integral for an awakening response (Buchanan et al., 2004; Wilhelm et al., 2007). Recently, it has also been suggested that the CAR is induced by a functional switch initiated by the process of awakening and involving both the hippocampus and the light-sensitive suprachiasmatic nucleus, responsible for bodily circadian rhythms (Clow et al., 2010). Whereas hippocampal inhibition is active during the pre-awakening period, this inhibition is switched off as a result of awakening and simultaneously, the suprachiasmatic nucleus switches from decreasing to increasing adrenal ACTH sensitivity by extrapituitary pathways (Clow et al., 2010), thus interacting in stimulating the cortisol response to awakening. This may be an explanation for the divergent heritability estimates reported for morning and evening cortisol levels. In a mixed child-adult sample (age range 8–64 years), Wüst and coworkers (Wust et al., 2000a) found a substantial genetic influence on the CAR but not on cortisol levels at awakening or during the later part of the day, suggesting that the CAR, as a distinct aspect of the diurnal cortisol regulation, is the phenotype under strong genetic influence rather than the basal cortisol levels. Unfortunately, the heritability of CAR has not been studied specifically in children, only one pediatric twin study in school-aged children (Bartels et al., 2003a) has been able to explore the genetic and environmental impact on both morning and evening cortisol levels, and only one study, in infants, has examined heritability for cortisol at different parts of the morning (Ouellet-Morin et al., 2009). Little is thus known about whether the heritability of cortisol levels in children differs with respect to the circadian rhythm.

The aim of the present study was to examine the relative contributions of genetic and environmental influences on different parts of the circadian cortisol rhythm in twin pairs aged 9–16 years, including the CAR and early morning and bedtime cortisol levels.

Methods

Parents of all Swedish 9 and 12-year-old twins were traced through the Swedish Twin Registry and contacted over the phone as part of the Child and Adolescent Twin Study in Sweden (CATSS) for interviews that screened for several somatic health (e.g., asthma, allergies, diabetes) and mental health problems (Anckarsäter et al., 2011). The study started in 2004 and the response rate of the telephone interview was 80% (Anckarsäter et al., 2011). Interviewers from a professional company carried out the interviews after a brief introduction in child and adolescent psychiatry and twin research.

In the present study the first 65 consecutive twin pairs in whom at least one of the twins was screen-positive for ADHD ('ADHD group'), according to validated algorithms on the instrument used — the Autism – Tics, AD/HD and other Comorbidities (including anxiety and depression) Inventory: A-TAC (Larson et al., 2010) — as well as the first 254 consecutive twin pairs where both twins were screen-negative for any mental disorder ('healthy group'), were included. The purpose of this was to have a case-control design for investigating diurnal cortisol patterns in children with ADHD compared to normal children (manuscript in preparation), but in this article the sample is used for studying heritability. One hundred and sixty-five healthy twin pairs (51%) and 45 ADHD twin pairs (69%, including 48 ADHD screen-positive children), participated.

Zygosity was determined by an algorithm established by discriminant analyses on 281 twin pairs with zygosity confirmed by 48 polymorphic DNA-markers. This algorithm correctly classified more than 95% of the twin pairs. Among the 151 twin pairs with known zygosity, 77 were monozygotic (MZ, 53% males) and 74 were same-sexed dizygotic (DZ, 55% male–male twin pairs). Out of the 10 twin pairs who were excluded due to unknown zygosity, four pairs included at least one ADHD screen-positive child.

Procedure and Cortisol Analysis

The twins and their parents were contacted per mail, providing written information about the present study, together with a DVD instruction film on how to perform the saliva sampling for cortisol showing that the child

should remain in bed when the first saliva sample was taken, that the next sample should be taken 30 minutes later, and the last sample at bedtime. Parents and children signed informed consent and the saliva sampling was completed at home. The children were instructed not to engage in any strenuous activities, eat or drink for one hour preceding the saliva samplings. Supervised by a parent, the children were allowed to handle the collecting tubes by themselves. They were instructed not to touch the cotton swab. The swab was soaked with saliva during approximately 1–2 min in the mouth. Samples were taken immediately after awakening ('AWAKENING'); 30 min post-awakening ('+30 min'); and at bedtime ('BEDTIME', approximately at 2100 h). Cortisol Awakening Response ('CAR') was operationalized as the change in concentrations from awakening to +30 min.

The saliva sampling was done on two consecutive days (the same days for both siblings) when the children attended school (Monday and Tuesday). The samples were placed in protective casings and mailed on Wednesday morning, reaching the laboratory on the following Thursday. They were thus taken care of within 36 hours after the last sampling time, and were centrifuged and frozen at -20 degrees Celsius. For saliva collection, a commercial Salivette® (Sarstedt, Nümbrecht, Germany) tube containing a cotton wool swab was used. The saliva cortisol concentrations were determined by a commercial enzyme immunoassay (EIA) method (Salivary Cortisol Enzyme Immunoassay Kit, Salimetrics LLC, USA). The total precision of the method was *CV* (coefficients of variation) = 14.4% at 3 nmol/L and *CV* = 8.5% at 25 nmol/L (N = 368). Cortisol concentrations were calculated as mean values for the same time points on the two days and are presented as nmol/L.

The mean (range, *SD*) age of the children at cortisol sampling was 12.7 (9–16, 1.68) yr, since (because of practical circumstances of the study) there was a period between the initial contact with the families and the cortisol sampling. Eight children used corticosteroid spray medication, one used steroid ointment and none oral corticosteroids. Two children took stimulants. One child had diabetes, two seizures, and, according to parent report, 109 children had or had had atopic disease symptoms (asthma, hay fever, eczema or food allergy). Nineteen children had a clinical psychiatric diagnosis (nine ADHD, eight mild mental retardation, two autism spectrum disorder). There were no significant influences between children with/without a diagnosis and thus all children with a diagnosis were included in the final analysis. For a discussion of the group of twins that screened positive for ADHD, see statistical analysis below.

Ethical Considerations

Written informed consent was given by parents and children after written and oral information was provided. The study protocol accorded with the Helsinki declaration and was approved by the ethical review board of Karolinska Institute (D-nr 03–672) and by the regional ethical board of Linkoping University (D-nr M162-05).

Statistical Analyses

The twin method relies on the different level of genetic relatedness between MZ twin pairs and DZ twin pairs. MZ twins are genetically identical, whereas DZ twins share on average 50% of their segregating genes (Plomin et al., 2001). We used Mx (Neale, et al., 2003), a structural equation-modeling program, to perform twin analyses by the method of raw maximum-likelihood estimation. Confidence intervals (CI, 95%) were calculated for all parameter estimates.

Twin correlations (i.e., within-twin pair maximum-likelihood correlations) were used to measure the similarity between twins. Comparisons of twin correlations for MZ and DZ twin pairs provide information about the importance of additive genetic factors, shared environmental factors (environmental influences that make twin siblings similar to each other) and non-shared environmental factors (environmental influences that make twin siblings different from each other).

Univariate twin models were fitted to the data in order to decompose the phenotypic variance in AWAKENING, +30 min, CAR and BEDTIME into its additive genetic (A), shared environmental (C) and non-shared environmental (E) components. For each of the cortisol measures, a full univariate ACE model was compared against the nested and more parsimonious AE, CE and E models. Goodness of fit for the different twin models was assessed by a likelihood-ratio χ^2-test, which is the difference between -2 log likelihood (-2 ll) of the full model from that of the restricted model. This difference is distributed as a χ^2. The degrees of freedom (*df*) for this test are equal to the difference between the number of estimated parameters in the full model and that of the restricted model. In addition to the likelihood-ratio χ^2-test, Akaike's information criterion (AIC = χ^2 - 2 × *df*) was computed. A lower AIC value indicates better fit of the model to the observed data.

One of the assumptions of twin modeling is that the data are normally distributed. Skewness and kurtosis showed that AWAKENING, +30 min and CAR were approximately normally distributed, so no transformation was needed for these variables. Because BEDTIME was positively skewed (Skewness = 4.2; Kurtosis = 21.36) raw scores were first normalized, then standardized to unit variance in the SAS 9.1.3 using the RANK and STANDARD procedures (34), which reduced the skewness (Skewness = 0.00; Kurtosis = -1.20) of the distribution.

We also explored potential differences between the group of twins that screened positive for ADHD (i.e., 41 twin pairs) and the healthy group (i.e., 110 twin pairs without indications of any mental disorder according to the A-TAC interview) across the four cortisol measures. Since the ADHD discordant twin pairs may bias the

genetic and environmental estimates, all twin models were re-fitted after excluding these pairs. Almost identical results were obtained, suggesting that this bias is of very limited importance.

Results

Girls had significantly higher cortisol levels at AWAKENING and +30 min, as well as a higher CAR, while BEDTIME levels were similar across sex (Table 1). We found no evidence for differences in means or variances between MZ and DZ twin pairs. Significant correlations were observed between age and the cortisol measures (AWAKENING: r = -0.17; +30 min: r = -0.18; and BEDTIME: r = 0.32). All subsequent twin analyses were adjusted for sex and age. Cortisol medication was not included as a covariate as model-fitting analyses showed that it could be omitted from the models without a significant reduction in fit.

Twin correlations for each of the cortisol measures are shown in Table 2, separately for sex and combined. Twin correlations could be constrained to be equal across sex without a significant loss in fit, thus indicating that the genetic and environmental contribution to the cortisol measures were similar for males and females. MZ correlations were consistently higher than DZ correlations, except for BEDTIME, suggesting genetic influences on the morning cortisol measures. Almost identical MZ and DZ correlations for BEDTIME instead suggest a strong effect of shared environment influences. All MZ correlations were less than 1, suggesting non-shared environmental influence on all cortisol measures.

Table 3 displays the model fitting results of the univariate models for AWAKENING, +30min, CAR and BEDTIME. The associated parameter estimates along with 95% confidence intervals are shown in Table 4. The AWAKENING measure showed a significant worsening in fit when both genetic (A) and shared environmental factors (C) were dropped from the model, indicating that familial factors are important. Although the pattern of twin correlations and the parameter estimates from the full ACE model suggest that these familial influences was due to both genetic (29%) and shared environmental influences (26%), the relatively small sample size of the present study the did not provide sufficient statistical power to distinguish between genetic (i,e., power to detect A was estimated as 39% in the present full ACE model) and shared environmental (i,e., power to detect C was estimated as 15% in the present full ACE model) influences. For CAR, the pattern of twin correlations, the parameter estimates from the full ACE model and the pattern of AIC values suggest that the AE model was the preferred model, although statistical power is insufficient. The parameter estimates from the AE model show that the additive genetic factor explained 50% of the variance in CAR. For +30min, the shared environmental factor could be constrained to zero without a significant loss in fit. The parameter estimates from the best-fitting AE model show that the additive genetic factor explained 60% of the variance, whereas the remaining part (40%) was due to non-shared environmental influences. For BEDTIME, the additive genetic factor could be constrained to zero without a significant loss in fit. The parameter estimates

TABLE 1

Salivary Cortisol nmol/l

	Total		Boys		Girls		
	Mean (*SD*)	*n*	Mean (SD)	*n*	Mean (*SD*)	*n*	*p*
AWAKENING	5.89 (2.79)	301	5.48 (2.45)	163	6.38 (3.09)	138	.006
+30 min	10.41 (4.52)	301	9.16 (3.55)	163	11.88 (5.08)	138	< .001
CAR	4.52 (3.83)	300	3.71 (3.27)	162	5.49 (4.20)	138	< .001
BEDTIME	1.04 (1.38)	302	1.02 (1.46)	164	1.07 (1.30)	138	n.s.

TABLE 2

Sex- and Age-Adjusted Twin Correlations (i.e., Within-Twin Pair Maximum-Likelihood Correlations) for Cortisol Measures.

	Combined MZ twins	Combined DZ twins	Male MZ twins	Male DZ twins	Female MZ twins	Female DZ twins
AWAKENING	0.57 (0.42,0.69)	0.38 (0.13,0.56)	0.65 (0.47,0.78)	0.29 (-0.06,0.56)	0.48 (0.22,0.66)	0.48 (0.12,0.70)
+30 min	0.63 (0.50,0.74)	0.27 (0.02,0.47)	0.71 (0.53,0.81)	0.22 (-0.12,0.49)	0.55 (0.30,0.72)	0.34 (-0.02,0.60)
CAR	0.53 (0.31,0.69)	0.34 (-0.04,0.60)	0.47 (0.18,0.67)	0.23 (-0.11,0.50)	0.51 (0.34,0.64)	0.28 (0.03,0.48)
BEDTIME	0.56 (0.39,0.69)	0.52 (0.34,0.65)	0.52 (0.25,0.70)	0.53 (0.28,0.69)	0.60 (0.36,0.76)	0.51 (0.21,0.70)

from the best fitting CE model show that the shared environmental factor explained 54% of the variance, and that the remaining part of the variance (46%) was due to the non-shared environmental factor.

Phenotypic correlations for the three cortisol samples were 0.54 (0.43, 0.61) for AWAKENING and +30min, 0.16 (0.03, 0.28) for AWAKENING and BEDTIME and finally 0.06 (-0.06, 0.18) for +30min and BEDTIME. More specific examinations of whether genetic influences on cortisol levels in the morning are distinct from those that influence evening levels would require multivariate twin model fitting using all three cortisol measures. However, the low correlations between morning and evening cortisol levels in combination with the relatively small sample size constrain possibilities to conduct multivariate twin analyses with sufficient power.

TABLE 3

Univariate Model Fitting Results.

			Fit of model compared to saturated model			
Model	-2LL	df	Diff χ^2	diff-df	*p*	AIC
AWAKENING						
ACE	1417.781	296				
AE	**1418.952**	**297**	**1.171**	**1**	**0.279**	**-0.829**
CE	**1419.288**	**297**	**1.508**	**1**	**0.220**	**-0.492**
E	1461.132	298	31.642	2	0.000	39.351
+30 min						
ACE	1687.733	296				
AE	**1687.733**	**297**	**0.000**	**1**	**Inc.**	**-2.000**
CE	1694.404	297	6.672	1	0.010	4.672
E	1732.677	298	44.944	2	0.000	40.944
CAR						
ACE	1609.512	295				
AE	**1609.528**	**296**	**0.016**	**1**	**0.898**	**-1.984**
CE	1612.698	296	3.186	1	0.074	1.186
E	1638.709	297	29.197	2	0.000	25.197
BEDTIME						
ACE	797.280	297				
AE	803.210	298	5.930	1	0.015	3.930
CE	**797.479**	**298**	**0.199**	**1**	**0.655**	**-1.801**
E	849.728	299	52.448	2	0.000	48.448

Note: Best-fitting model indicated in bold.

TABLE 4

Parameter Estimates With 95% Confidence Intervals (CI) From Univariate Models for Each of the Cortisol Measures.

Model	A	C	E
Awakening			
ACE	.29 (0.00; 0.65)	.26 (0.00; 0.58)	.45 (0.34; 0.61)
AE	**.56 (0.42; 0.67)**	**—**	**.44 (0.33; 0.58)**
CE	**—**	**.49 (0.36; 0.60)**	**.51 (0.40; 0.64)**
E	—	—	1.00 (1; 1)
+30 min			
ACE	.60 (0.14; 0.71)	.00 (0.00; 0.40)	.40 (0.29; 0.54)
AE	**.60 (0.46; 0.71)**	**—**	**.40 (0.29; 0.54)**
CE	—	.48 (0.34; 0.59)	.52 (0.41; 0.66)
E	—	—	1.00 (1; 1)
CAR			
ACE	.47 (0.00; 0.63)	.03 (0.00; 0.45)	.50 (0.37; 0.67)
AE	**.50 (0.34; 0.63)**	**—**	**.50 (0.37; 0.66)**
CE	**—**	**.40 (0.26; 0.52)**	**.60 (0.48; 0.74)**
E	—	—	1.00 (1; 1)
BEDTIME			
ACE	.09 (0.00; 0.51)	.47 (0.10; 0.64)	.44 (0.31; 0.58)
AE	.60 (0.47; 0.71)	—	.40 (0.29; 0.53)
CE	**—**	**.54 (0.42; 0.64)**	**.46 (0.36; 0.58)**
E	—	—	1.00 (1; 1)

Note: Best-fitting model indicated in bold.

Discussion

Main Results

The main finding of the present study is that the heritability estimates vary across the different parts of the circadian cortisol rhythm. Heritability was strongest for cortisol levels +30 min post-awakening, with no influence of shared environment. These findings add support to one (Bartels et al., 2003a) of two (Bartels et al., 2003a; Ouellet-Morin et al., 2009) previous twin study addressing different parts of the diurnal rhythm in children, though the latter only examined morning cortisol levels and focused on the relation to family adversity. In contrast, we found that the familial resemblance of evening cortisol levels was mainly explained by environmental effects. CAR, operationalized as the change in concentrations from awakening to the +30 min sampling, was genetically influenced to a similar extent as the +30 min. Cortisol levels immediately after awakening had a considerable part explained by genetic effects, but also by shared and non-shared environmental influences. The finding of a substantial genetic influence on saliva cortisol levels in the morning but not in the evening is in agreement with the results from a number of twin studies (Bartels et al., 2003a; Bartels et al., 2003b; Kupper et al., 2005; Ouellet-Morin et al., 2009; Schreiber et al., 2006). Our findings of little environmental influence on CAR, some on cortisol at awakening and much on evening levels might imply two genetic regulation patterns, one specifically for CAR, and one for the circadian rhythm proper.

Underlying Mechanisms

Although confidence intervals were large, our results of different patterns of heritability estimates for cortisol samples taken merely 30 min apart are noteworthy.

Similar to our findings, other research groups demonstrate that genetic (and environmental) contributions to cortisol levels vary as a function of the time of the day, with genetic dominance in the morning but not in the evening in adults (Kupper et al., 2005), 12-year-olds (Bartels et al., 2003a) and 6-month-old infants Ouellet-Morin (Ouellet-Morin et al., 2009). Wüst et al. (2000a) also showed a significant genetic impact on the CAR. Although morning values showed the highest heritability, one should take into account possible genetic and environmental interaction (Bartels et al., 2003b). Even when genetic factors account for most of the variance in morning cortisol levels, this is more likely to be expressed in infants with family adversity (Ouellet-Morin et al., 2009). Adversity factors, representing environmental contribution, may influence morning cortisol levels when becoming chronic (Miller et al., 2007). Furthermore, there is growing evidence of an increased CAR in individuals with chronic stress (Chida & Steptoe, 2009; Wüst et al., 2000a). Thus, morning cortisol levels and the CAR appear to be more clearly influenced by genetic factors than cortisol levels during the rest of the day, although even early day cortisol levels also are susceptible to chronically burdensome life demands.

According to Edwards and co-workers (Edwards et al., 2001) salivary cortisol secretion over the day can be divided into two phases: the CAR and the subsequent period of decline in cortisol across the rest of the day. This was also stated by Oskis and the Clow-team (Oskis et al., 2009) who support that CAR is distinct from the basal circadian rhythm of cortisol secretion, and supported by the findings of Wilhelm et al. (2007), who demonstrate that CAR is distinct from the diurnal variation, and rather an additional phenomenon associated with awakening. Cortisol activity might be regulated by different structures at awakening versus later during the morning (Kupper et al., 2005). The regulation of CAR is believed to be dependent on hippocampus to a greater degree than the other parts of the circadian rhythm (Bruehl et al., 2009; Buchanan et al., 2004; Herman et al., 2005; Pruessner et al., 2007). It is tempting to suggest that cortisol at +30 min, and correspondingly the CAR, could be viewed as measures of the activation of the HPA-axis in response to the activities of a new day (Wilhelm et al., 2007), and that cortisol levels at awakening rather relates to the circadian rhythm proper. It is thus possible the reported discrepant heritability estimates for the cortisol measures sampled at different times of the day are rooted in the distinct neurobiological regulatory mechanisms of cortisol across the circadian rhythm.

Sex Differences

The girls in our study exposed significantly higher morning cortisol levels and CAR compared to the boys, but similar bedtime values. Higher morning, but not evening, cortisol levels in girls has been reported previously in children (Netherton et al., 2004; Rosmalen et al., 2005), and a more pronounced and prolonged CAR has been described in adult women compared to men (Pruessner et al., 1997; Wright & Steptoe, 2005; Wüst et al., 2000b). As results suggest that puberty involves distinct changes in the cortisol regulation after awakening specifically in girls (Netherton et al., 2004; Oskis et al., 2009), pubertal maturation may play a key role in the development of these sex differences. However, the impact of sex on CAR is said to be rather small (Pruessner et al., 1997; Wüst et al., 2000a), and several studies have reported similar cortisol levels in boys and girls (Gröschl et al., 2003, Kiess et al., 1995, Knutsson et al., 1997).

Methodological Aspects

Methodological strengths of the study are the epidemiologic sample of children, that the socioeconomic distribution (data not shown) was similar to Sweden as a whole, indicating that the investigated children could be considered as representative for Sweden, and a two-day saliva sampling protocol. However, the relatively small sample size of the present study did not provide sufficient statistical power to distinguish between the

additive genetic and shared environmental factors for some of the measures. Thus, the 95% CI for some of the estimates are broad, which calls for caution in interpretations. Socioeconomic factors did not influence the heritability measures, but, as reported previously, had an impact on cortisol levels (Gustafsson et al., 2010a). Unfortunately, we did not collect data on pubertal stage or menarche, something that could have been of value for the interpretation of the findings. The fact that we found no significant influences on cortisol levels between children taking cortisol medication or not, and between children with/without a diagnosis could of course be explained by the relatively small sample and limited power to detect such influences. There was also a low systematic control of adherence to the saliva sampling protocol, which could introduce random as well as systematic error (Kudielka et al., 2003). This is particularly important for the samples in the morning, when cortisol levels change rapidly and also are influenced by the subjective assessment of awakening (Dockray et al., 2008). Electronic monitoring of sampling (Broderick et al., 2004) and objective measures of awakening (Dockray et al., 2008) would have been preferable to increase the control of sampling compliance. Another limitation is that we did not assess the full CAR (expanding the time to +45min and +60min), and that we collected only a single evening level. A general problem with evening levels is that on average they are below the optimal measurement range of most salivary cortisol assays, resulting in higher coefficients of variations, as in the present study.

Conclusion

In conclusion, our findings are in line with and extend previous twin studies of genetic and environmental effects on cortisol levels, indicating that inter-individual variations in cortisol secretion in children is influenced by both environmental and genetic factors and that these influences affect specific parts of the diurnal rhythm. Environmental factors have a profound effect on evening cortisol and possibly some effect on cortisol levels at awakening, while genetic factors seem to have a more exclusive influence on the cortisol awakening response and less on levels immediately at awakening. These findings provide guidance for future studies by indicating that heritability might be particularly important for the cortisol awakening response, in contrast to the circadian rhythm.

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