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# Co-Twin Relationship Quality as a Moderator of Genetic and Environmental Factors on Urinary Cortisol Levels among Adult Twins

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## Abstract

Previous research has indicated that genetic and environmental factors shape physiological activity. Cortisol levels, in particular, have received significant attention, with studies indicating substantive heritability estimates across various sampling techniques. A related line of research has indicated that genetic and environmental factors that explain variability in cortisol levels may vary across context and experiences by way of gene-environment interactions (G×Es). Despite these findings, a limited number of studies have examined the extent to which interpersonal relationships may operate as a moderator. The current study focused on co-twin relationship quality as a source of moderation, as twins are more likely to have contact with one another and to form close, interpersonal relationships with their co-twin relative to singleton siblings. Using a sample of 298 adult twins from the National Survey of Midlife Development in the United States (MIDUS), we examined the extent to which genetic and environmental factors that explain variability in urinary cortisol levels varied across levels of co-twin relationship quality. The heritability of cortisol levels was greater and nonshared environmental influences were lower at greater levels of relationship quality. These findings suggest that the heritability of cortisol may vary across context, and positive relationships with others may moderate such factors.

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AUTHOR CONTRIBUTIONS

J.A.S developed the study concept, conducted data analyses and drafted the manuscript. S.J. provided critical revisions of the manuscript and assisted in the interpretation of findings. J.L.C. and D.A.G suggested study design revisions, assisted in drafting the manuscript as well as interpreting key findings. All authors approved the final version of the manuscript.

**Keywords**

gene-environment interaction; urinary cortisol; interpersonal relationships

## INTRODUCTION

Cortisol, a glucocorticoid hormone secreted by the outer region of the adrenal gland and primary product of the hypothalamic-pituitary-adrenal (HPA) axis, has been the subject of a significant amount of scholarship focused on better identifying psychophysiological responses to environmental stressors (De Kloet et al., 1999; Weiner, 1992). Acute cortisol reactivity to environmental challenges is largely adaptive (Rovida et al., 2015), but extended periods of increased cortisol secretion has been found to result in increased risk of mental and physical health problems (Chrousos, 2000; McEwen and Seeman, 1999). Cortisol levels can be measured in a variety of samples, including blood, saliva, urine, and hair (Kirschbaum and Hellhammer, 1994; Murphy, 2002; Short et al., 2016; Stalder and Kirschbaum, 2012), but the resulting estimates reflect different aspects of cortisol production and temporal patterns. Cortisol concentrations in blood and saliva reflect more contemporaneous measures of circulating cortisol (Kirschbaum and Hellhammer, 1994), while urinary sampling provides an integrated measure of total cortisol production across the collection period (typically 12 or 24 hours) (Murphy, 2002), and hair cortisol concentration can provide estimates of cortisol levels for up to six months (Kirschbaum et al., 2009). Cortisol concentrations can be assessed in all of these samples, but studies indicate that sampling techniques may tap unique aspects of cortisol production and regulation over time (Short et al., 2016). The current study examines urinary cortisol, as this measure taps total cortisol production and few studies have examined genetic and environmental influences on urinary cortisol (Inglis et al., 1999).

Individual differences in cortisol secretion has been linked to a wide range of environmental stimuli, with previous studies recognizing that a significant portion of the overall variance in cortisol levels is explained by genetic factors (Bartels et al., 2003b; Inglis et al., 1999; Riese et al., 2009; Rietschel et al., 2017; Tucker-Drob et al., 2017). Heritability estimates of salivary cortisol levels range between 45% and 72%, with an average heritability estimate of 62% (Bartels et al., 2003b). Heritability estimates of urinary cortisol levels are similar to the average heritability of salivary levels and have been reported to be approximately 59% (Inglis et al., 1999). Despite the consistency of these findings, subsequent studies have revealed additional information surrounding genetic factors and cortisol levels. Samples capturing cumulative cortisol production, such as hair cortisol concentration, yield greater heritability estimates (Rietschel et al., 2017; Tucker-Drob et al., 2017), indicating that genetic factors tend to be more centrally implicated in the development of long-term patterns of cortisol secretion. Similar findings have been observed for salivary measures of cortisol awaking response (Bartels et al., 2003a; Van Hulle et al., 2012) and morning cortisol levels (Riese et al., 2009), with lower heritability estimates for cortisol levels throughout the day and evening (Kupper et al., 2005).

In addition to independent associations between genetic and environmental factors and cortisol levels, previous studies have found evidence of gene-environment interactions (G×Es) wherein heritability estimates of cortisol levels vary across environmental context (or vice versa). Genetic and environmental influences on overall cortisol levels and reactivity vary across levels of family-level adversity (Ouellet-Morin et al., 2008), childhood maltreatment (Gerritsen et al., 2017), and socioeconomic status (Tucker-Drob et al., 2017). Similar environments have also been examined within the context of candidate gene-environment interactions (cG×E), in which the moderating effects of measured genetic polymorphisms and environments are estimated (Cicchetti and Rogosch, 2012; Feder et al., 2009; Frigerio et al., 2009; Mueller et al., 2011; Tyrka et al., 2009). While the majority of both sets of studies have focused on adverse environmental context (in line with the diathesis-stress hypothesis), additional studies have emphasized positive environments (Cicchetti and Rogosch, 2012; Frigerio et al., 2009). This possibility aligns with findings from previous studies linking environments like more secure attachment and relationship functioning to the down-regulation of emotional and physiological responses to encountered stressors (Adam and Gunnar, 2001; Papp et al., 2009; Powers et al., 2006; Smyth et al., 2015). Previous studies have also reported evidence of synchrony in physiological responses, wherein increased contact and social attachment between members of a given dyad may result in increased coordination of physiological system functioning (Ha et al., 2016; Ha and Granger, 2016; Rankin et al., 2018; Sbarra and Hazan, 2008). This process, frequently referred to as *attunement*, suggests that interpersonal relationships contribute to individual differences in the functioning of physiological systems including the HPA axis.

One way relationships may be associated with physiological system functioning is by moderating underlying genetic factors, wherein greater exposure to such relationships may allow for greater levels of underlying genetic expression and lead to greater similarity in cortisol production among siblings. For example, previous studies have suggested that genetic influences may be greater in less restrictive environments, as such environments provide a greater diversity of options allowing individuals to select those options that better suit underlying genetic expression (Dick et al., 2007, 2001). Importantly, this possibility does not necessarily imply that cortisol levels will decrease or increase as a result of attunement-based processes, but rather, levels will better reflect underlying genetic predispositions (for better or worse). This pattern of moderation also aligns with Bronfenbrenner and Ceci's (1994) bioecological model, where genetic influences are greater in enriched environments, as such environments are expected to minimize individualized variability in phenotypes. Alternatively, the *social push* perspective recognizes the possibility that environmental influences may become so adverse, they become more pronounced than underlying genetic predisposition (Burt and Klump, 2014; Raine, 2002). Collectively, these perspectives demonstrate the importance of considering the ways in which a given source of environmental influence may account for individual differences in a given phenotype via a moderated pathway as opposed to a more direct association.

One potential interpersonal relationship that may moderate genetic factors is the relationship between co-twins. Previous studies have found reported the novelty of co-twin relationships, noting that twins are more likely to have contact with one another (Neyer, 2002), form

secure attachment bonds with their co-twin (Tancredy and Fraley, 2006), and rely on their co-twin for safety and security compared to singleton siblings (Fraley and Tancredy, 2012). These findings, coupled with those highlighting the importance of exposure to positive interpersonal relationships in regulating HPA axis activity, suggest that the impact of co-twin relationship quality may contribute to individual differences in cortisol levels via the moderation of genetic and environmental factors. While such factors may operate independently as environmental sources of influence, findings from previous studies examining G×Es involving both latent and measured sources of genetic factors, provide additional evidence of a moderating effect. The current study aims to examine this possibility by employing a sample of twins from the National Survey of Midlife Development in the United States (MIDUS).

# METHODS

## PARTICIPANTS AND PROCEDURES

The current study analyzes data from the MIDUS, a longitudinal study that includes a nationally representative sample of adults from the United States (Brim et al., 2004, 1996). The first wave of data collection (MIDUS I) was completed between 1995 and 1996 ($N$ = 7,108). The second wave of data collection (MIDUS II) was conducted between 2004 and 2006 and had a 70% retention rate ($N$ = 4,963). A subsample of MIDUS II participants ($n$ = 1,255) were also asked to participate in the Biomarker Project, which consisted of an extensive battery of physical and mental health assessments carried out over two days at one of three General Clinical Research Centers. Trained medical professionals collected information on a wide range of factors related to medication use, psychosocial experiences, and sleep quality along with 12-hour urine samples and fasting blood draws (Love et al., 2010).

The MIDUS also oversampled twins ($n$ = 1,914 for MIDUS I and $n$ = 1,484 for MIDUS II). For families with more than one twin pair, all pairs that agreed to participate were recruited into the MIDUS sample. Zygosity was assessed during MIDUS I interviews using a confusability index (Rietveld et al., 2000). A subsample of twins that participated in the MIDUS II, also participated in the Biomarker Project (Love et al., 2010). Of the 388 twins that participated in the Biomarker Project, zygosity could not be determined for 3.61% ($n$ = 14) and an additional 20.62% ($n$ = 80) of participants' co-twins were not included in the Biomarker Project, resulting in a final analytic sample of 298 individuals consisting of both monozygotic (MZ; $n$ = 156) as well as same-sex ($n$ = 86) and opposite sex ($n$ = 56) dizygotic (DZ; $n$ = 142) twins. The biomarker twin sample had lower overall urinary cortisol levels ($t(1253) = 4.89$, $p < .001$) and fewer males ($\chi^2(1) = 4.78$, $p = .03$) compared to non-twin biomarker subsample, but there were no significant differences between the two subsamples for age ($t(1253) = 1.29$, $p = .20$), race ($\chi^2(1) = 3.25(1)$, $p = .07$), and economic adversity ($\chi^2(2) = 1.38$, $p = .50$). All MIDUS participants provided informed consent and all data collection procedures were reviewed and approved by the Education and Social/Behavioral Sciences and the Health Sciences Institutional Review Boards at the University of Wisconsin-Madison. All methods were performed in accordance with the

relevant guidelines and regulations approved by the Institutional Review Boards mentioned above.

## MEASURES

**Urinary Cortisol.—**Twelve-hour (7:00 pm to 7:00 am) urine samples were collected from participants by trained staff at each of the General Clinical Research Centers. Urinary cortisol was assessed using enzymatic colorimetric assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Deuterated cortisol [d(3)-cortisol] was added to a 0.1-mL urine specimen as an internal standard. Cortisol was extracted from samples using on-line turbulent flow high-pressure liquid chromatograph (HPLC) and analyzed by liquid chromatography-tandem mass spectrometry using multiple reaction monitoring in positive mode. The inter-assay coefficient of variance for cortisol was 6.1% with a reference range of 3.5-45 μg/day.

Creatinine was also assessed using enzymatic colorimetric assay in which creatininase, creatinase, and sarcosine oxidase (which react with creatinine and produce hydrogen peroxide) were employed. The liberated hydrogen peroxide is measured via a modified Trinder reaction using a colorimetric indicator. Optimization of the buffer system and colorimetric indicator enables the creatinine concentration to be quantified. The inter-assay coefficient of variance for creatinine was 0.85% with a reference range of 1-2 g/day for males and 0.6-1.8 g/day for females (Ryff et al., 2011). Raw cortisol values were divided by creatinine levels to adjust for overall urine concentration and volume. The resulting measures were winsorized to three standard deviations of each participant's mean to address outliers and then log-transformed to reduce levels of right skew.

**Co-Twin Relationship Quality.—**Co-twin relationship quality was measured using items from the twin zygosity screening survey collected via telephone during the MIDUS I interview. Participants were asked four questions regarding their interactions with their co-twin: 1) how much does your twin understand your feelings; 2) how much do you rely on your twin if you have a serious problem; 3) how much can you open up to your twin if you need to talk about your worries; and 4) when you have a problem, how much of the time do you turn to your twin for advice or help. The first three items had response categories ranging between 1 (not at all) to 4 (a lot), while the fourth item had response categories ranging between 1 (never or hardly never) and 5 (all of the time). The four items were summed ($\alpha$ = .64), with greater values reflecting greater co-twin relationship quality. Individual co-twin scores from each family were averaged to reflect a family-level measure of co-twin relationship quality.

**Statistical Covariates.—**Four statistical covariates were included in the estimated analytic models. First, age was self-reported during the MIDUS II interview and was measured continuously in years ($M$ = 52.55, $SD$ = 11.46). Sex was self-reported during the Biomarker Project and coded dichotomously such that 0 = *female* (61.74%) and 1 = *male* (38.26%). Race was self-reported during the MIDUS II interview. Since the final analytic sample predominately identified as Caucasian (94.30%), the measure was dichotomized with the remaining categories collapsed into a single category representing all other races

(5.70%). Finally, an economic adversity measure was used to tap socioeconomic status using a single item from the MIDUS II interviews in which participants were asked whether they had enough money to meet their needs. The response categories were coded 1 = more than enough, 2 = just enough, and 3 = not enough.

## ANALYTIC APPROACH

The analysis was carried out in three steps. First, a series of cross-trait and cross-twin correlations were estimated. The cross-trait correlation coefficients would reveal the extent to which co-twin relationship quality and cortisol levels covary. This step is necessary as a significant cross-trait correlation coefficient would provide preliminary evidence of a gene-environment correlation (*r*GE), which refers to a set of phenomena in which underlying genetic predisposition manifests as an environmental factor (Knopik et al., 2017; Scarr and McCartney, 1983). While there are three primary sources of *r*GE, active *r*GE is the most salient within the context of the current study, as this form of *r*GE occurs when individuals seek out environments that best match their underlying traits, which, in turn, are influenced at least in part by genes. These genetically-influenced selection processes may result in significant changes in the encountered environment due to human agency and individually-based preferences. This overlap in genetic and environmental factors may result in biased estimates, as variance attributed to environments is actually explained (at least in part) by genes. These conceptual issues can also result additional biases. Failing to control for an *r*GE may increase the likelihood of detecting a false positive when examining the presence of G×E. This bias stems from multicollinearity due to the correlation between genetic and environmental factors involved in both G×E and *r*GE (Purcell, 2002). For these reasons, it is critical to account for *r*GE when examining a G×E. The cross-twin correlation is a preliminary estimate of the similarity of the urinary cortisol measure across zygosity. A larger correlation coefficient for MZ twins relative to DZ twins would provide preliminary evidence of a significant heritability estimate.

The second step in the analysis involved the estimation of a series of specialized structural equation models (i.e., univariate biometric models) that decompose the variance in the urinary cortisol measure into three components: 1) additive genetic influences (A); 2) shared environmental influences (C); and 3) nonshared environmental influences (E). Figure 1 displays a path diagram of the univariate biometric model. A is estimated as the covariance between twins from the same family on the urinary cortisol measures. The resulting within-pair correlations were constrained in line with additive genetic theory and reflect the proportion of shared genes between twins, $r = 1.00$ for MZ twins and $r = .50$ for DZ twins. Since C is expected to vary between, but not within, pairs the resulting within-pair correlation was constrained to 1.00 for MZ and DZ twins. Finally, since nonshared environmental influences are expected to vary both within and between pairs resulting in behavioral differences (and measurement error), E is estimated as the residual variance unexplained by A and C.

The third and final step of the analysis involved the estimation of series of modified univariate biometric models that included interaction terms. As can be seen in Figure 1, the univariate biometric model permits the addition of interaction terms, which examine the

extent to which the main effects are moderated by a measured family-level environment (e.g., co-twin relationship quality or *RQ*) (Purcell, 2002). The modified biometric model allows for the estimation of a G×E ($a + \beta_a RQ$), alongside interactions involving shared environmental ($c + \beta_c RQ$) and nonshared environmental ($e + \beta_e RQ$) influences, wherein *RQ* is the family-level co-twin relationship quality measure. Positive and significant interaction terms would indicate that the proportion of variance in cortisol explained by the accompanying main effect was greater at greater levels of co-twin relationship quality. Alternatively, a negative and significant interaction term would indicate that the proportion of variance explained in urinary cortisol was lower at greater levels of co-twin relationship quality. While the current study is more focused on G×E, taking into account the potential presence of an *r*GE involving co-twin relationship quality and urinary cortisol levels is necessary, as failing to account for the co-occurrence of G×E and *r*GE may result in biased estimates. For these reasons, previous studies have recommended residualizing any covariance between the two examined measures prior to the estimation of a univariate model involving interaction terms (represented by path *s* in the figure; Purcell, 2002). Finally, the urinary cortisol measure and the co-twin contact measure were *z*-transformed prior to the estimation of the modified univariate biometric models.

All analyses were performed in M*plus* 8.1 (Muthén and Muthén, 2017). The statistical covariates were included in the means portion of the univariate biometric models as well as the extended univariate moderation models. Missing values were addressed with full information maximum likelihood estimation (FIML) and all biometric models were estimated with robust standard errors. Model fit was assessed using multiple indices including Satorra-Bentler scaled $\chi^2$, the comparative fit index (CFI; values ≥ .95 indicate a close fit and values ≥ .90 indicate an acceptable fit), the Tucker Lewis Index (TLI; interpreted similarly to the CFI), and the root mean square error of approximation (RMSEA; values < .05 indicate a close fit and values < .10 indicate an acceptable fit) (Hu and Bentler, 1999). For models that include interaction terms, traditional fit indices are not available, but nested models can be compared using a likelihood ratio test (LRT) (Purcell, 2002). The results of the LRT are distributed in $\chi^2$ units and a nonsignificant change in $\chi^2$ would indicate that the more restricted (or nested) model would not result in a significant loss of overall fit.

## RESULTS

Descriptive statistics for the final analytic sample are presented in Table 1. The overall sample was approximately 52 years old during the MIDUS II interviews ($M = 52.52$, $SD = 11.46$), predominately Caucasian (94.30%) and comprised of more females than males (61.74% females). Urinary cortisol measures (adjusted for creatinine levels, winsorized to three standard deviations of each participant's mean , and log-transformed) did not significantly differ between DZ twins ($M = 2.69$, $SD = .64$) and MZ twins ($M = 2.56$, SD = .69) ($t = 1.75(296)$, $p = .08$). Average family-level co-twin relationship quality was significantly greater among MZ twins ($M = 14.46$, $SD = 2.03$) relative to DZ twins ($M = 13.49$, $SD = 2.36$) ($t = 3.82(296)$, $p < .001$). These findings potentially violate an underlying assumption of univariate moderation models, as such models traditionally assume the mean and variance of the examined moderator are equivalent across zygosity. For these reasons,

a supplemental univariate moderation model was estimated in which the mean and variance of the co-twin relationship quality measure were allowed to vary freely across groups. The results (not presented, but available upon request) directly aligned with those from the primary analysis.

Cross-trait and cross-twin correlations were calculated, with the results presented in Table 2. The cross-trait correlations revealed a nonsignificant association for the full sample ($r$ = .08, $p$ = .19), as well as the DZ subsample ($r$ = .01, $p$ = .88), but a small correlation for the MZ twin subsample ($r$ = .19, $p$ = .02), providing preliminary evidence of an $r$GE between co-twin relationship quality and urinary cortisol levels. The results of the cross-twin correlations for the urinary cortisol measure indicated significant correlation coefficients for the full sample ($r$ = .25, $p$ = .001), as well as a larger cross-twin correlation coefficient for the MZ twin subsample ($r$ = .39, $p$ < .001) was relative to the DZ twin subsample ($r$ = .19, $p$ < .001), providing preliminary evidence of genetic factors explaining a significant portion of the variance in urinary cortisol levels. Finally, the cross-twin correlations for the co-twin relationship quality demonstrated general agreement between twins from the same family ($r$ = .59, $p$ < .001), and greater agreement among MZ twins ($r$ = .66, $p$ < .001) relative to DZ twins ($r$ = .51, $p$ < .001).

The results from the univariate biometric models are presented in Table 3. The baseline univariate model provided a close fit to the data ($\chi^2(27) = 19.29$; $CFI$ = 1.00; $TLI$ = 1.00; $RMSEA$ = .00), but the results of an LRT indicated that constraining the C parameter to zero did not worsen overall fit ($\chi^2(1) = .00$, $p$ = 1.00). Based on these results, an AE model was selected as the best-fitting, most parsimonious model. The results of the AE model yielded an estimate of .59 (95% $CI$ = .43; .75, $p$ <.001) for the A parameter and an estimate of .70 (95% $CI$ = .59; .81, $p$ < .001) for the E parameter. These estimates are unstandardized but can be converted to proportions by diving a squared coefficient by the summed squares of all coefficients. Converting the estimates to proportions revealed that approximately 41% (95% $CI$ = .23; .60, $p$ < .001) of the variance in urinary cortisol was explained by genetic factors, while the remaining 59% (95% $CI$ = .40; .77, $p$ < .001) of the variance was explained by nonshared environmental factors (and measurement error).

The next step of the analysis involved the estimation of a univariate moderation model, with the results presented in Table 3. The univariate moderation model was an AE model with interaction terms for both parameters ($\beta_A$ and $\beta_E$, respectively). Based on the results of the baseline univariate model, the C parameter (along with the accompanying interaction term, $\beta_C$) was omitted. The results revealed that the A parameter was significantly moderated by co-twin relationship quality ($\beta_A$ = .20, 95% CI = .02; .37, $p$ = .03) such that the heritability of urinary cortisol was greater at greater levels of co-twin relationship quality. The interaction involving the E parameter was significant and negative, indicating that at greater levels of co-twin relationship quality, the proportion of variance explained by nonshared environmental factors was lower ($\beta_E$ = −.16, 95% CI = −.23; −.09, $p$ < .001). To aid in the interpretation, the variance explained in urinary cortisol levels by A and E is plotted as a function of co-twin relationship quality in Figure 2.

A series of sensitivity analyses were also performed to examine the robustness of the findings. First, the final sample size ($N$ = 298 twins) is relatively modest, potentially yielding limited power to detect small effect sizes. In order to examine whether the findings reported in the primary analysis were sensitive to this limitation, the univariate moderation model was re-estimated using 10,000 bootstrapped samples (with replacement). Bootstrapping procedures are robust to many limitations that accompany limited statistical power (Fu et al., 2005; Mooney and Duval, 1993). The results of the supplemental model are presented in the accompanying online information, and directly aligned with the results of the primary analysis. The model revealed a significant and positive G×E ($\beta_A$ = .20, bias corrected bootstrapped 95% CI = .01; .38, $p$ = .04) and a negative and significant interaction between co-twin relationship quality and nonshared environments ($\beta_E$ = −.16, 95% CI = −.24; −.08, $p$ < .001).

Second, to minimize omitted variable bias, seven additional statistical covariates were considered. During the MIDUS II interview, participants were asked if they currently smoked cigarettes regularly and whether they exercised for at least 20 minutes three or more times per week with responses coded dichotomously (0 = no, 1 = yes). Alcohol consumption in the past month was self-reported with responses ranging between 1 (never) to 6 (everyday). Participants were also asked to report their overall physical health with response categories ranging between 1 (worst) and 10 (best). Depression was measured using the Center for Epidemiologic Studies Depression (CESD) scale and coded continuously. Body mass index (BMI) was calculated for all participants and measured continuously. Finally, self-reported medication use that has been previously found to influence cortisol (e.g., steroid-based medications, medications containing cortisone, antidepressants, birth control, and other hormonal medications) was coded dichotomously such that 0 = no medications reported and 1 = one or more medications reported. The zero-order correlations between the urinary cortisol measure and the additional covariates were calculated. Only the association between BMI and cortisol ($r$ = −.20, $p$ = .001) emerged as significant. Despite this finding, all biometric models were reestimated with a modified cortisol measure in which all variance explained by the above-mentioned covariates was removed. The results of these supplemental models directly aligned with the primary analysis. Based on these findings, and in the interest of model parsimony, these additional covariates were omitted from the primary analysis.

Third, the estimated extended univariate moderation models assume no covariance between the examined outcome and moderator variables, and, by extension, the lack of an $r$GE (Purcell, 2002). This assumption is addressed in the primary analysis by residualizing any covariance between co-twin relationship quality and urinary cortisol prior to estimation. In situations in which the examined moderator varies both within and between dyads, a modified bivariate Cholesky model, which simultaneously estimates $r$GE and G×E may also be appropriate (Purcell, 2002). A series of baseline bivariate Cholesky models revealed evidence of a small to moderately sized $r$GE ($r$GE = .38) between co-twin relationship quality and urinary cortisol. Additional models that estimated $r$GE and included interaction terms revealed a pattern of results that directly aligned with the results from the primary analysis, with a positive and significant interaction term for genetic factors ($\beta_A$ = .30, $p$ = .01), and a negative and significant interaction term for nonshared environmental factors

($\beta_E$ = −.14, $p$ = .002). It should be noted that these models include far more estimated parameters and, in turn, require greater levels of statistical power. Accordingly, these results should be interpreted with caution. A more detailed description of the estimated bivariate Cholesky models, as well as the results from the sensitivity analyses, is presented in the accompanying online information.

## DISCUSSION

The current study examined the role of co-twin relationship quality as a moderator of genetic and environmental factors on urinary cortisol levels. The results revealed three key findings. First, univariate biometric models revealed a heritability estimate of approximately 42%, while the remaining 58% of the variance was explained by nonshared environmental factors (along with measurement error). This finding is interesting, as the only previous study to examine the heritability of urinary cortisol, reported heritability estimates of .59 (Inglis et al., 1999). While the differences between these estimates are likely nonsignificant, the discrepancy warrants attention. These differences may stem from the fact that the urinary cortisol measures employed in the current study were adjusted for overall urine creatinine, while Inglis and colleagues (1999) examined total cortisol. Such an adjustment is important, as it accounts for differences in urine concentration and volume across the collection period, normalizing the examined analyte (Remer et al., 2008). In addition, previous studies have indicated that creatinine levels are also heritable, potentially contributing to inflated heritability estimates of cortisol that are not properly adjusted (Arpegård et al., 2015).

The second key finding to emerge stemmed from the cross-trait correlations and revealed that co-twin contact and urinary cortisol levels were not significantly associated in the full sample ($r$ = .08, $p$ = .19) or the DZ twin subsample ($r$ = .01, $p$ = .88), but was positively and significantly associated in the MZ twin subsample ($r$ = .19, $p$ = .02). These findings provide preliminary support for the presence of an *r*GE, wherein genetic factors that collectively explain variance in both urinary cortisol levels and co-twin relationship quality are correlated. Findings from supplemental analyses also revealed evidence of a small to moderately sized *r*GE (*r*GE = .38). The estimated univariate moderation models residualized any covariance between co-twin relationship quality and urinary cortisol prior to estimating any interaction terms, which would also include any covariance explained by *r*GE, to minimize bias stemming from confounding and multicollinearity. However, these findings have potential implications for studies reporting patterns of adrenocortical attunement within other dyads, particularly those that share genes, such as mothers and infants. These findings provide evidence of attunement stemming from similarity in genetically influenced traits, and subsequent responses, as opposed to strict environmentally-based regulatory processes, which have been previously hypothesized to drive such patterns (Gunnar and Donzella, 2002; Sbarra and Hazan, 2008). These findings should be interpreted with caution, however, as previous studies examining attunement-based processes have made relied primarily on salivary cortisol measures.

This distinction in samples is important as recent findings reported by Short and colleagues (2016) indicate significant variability across cortisol concentrations collected from a variety of sampling procedures. Despite significant levels of test-retest stability in both

hair cortisol concentration and one month integrated 24-hour urinary free cortisol, the correlation between the two measures was nonsignificant. This finding was attributed to the conversion of free cortisol to cortisone (Murphy, 2002; Short et al., 2016). While similar processes impact other sampling procedures (Stalder and Kirschbaum, 2012), there is some preliminary evidence that the conversion of cortisol to cortisone may vary across sampling techniques (Murphy, 2002). For these reasons, future research would benefit from replicating these findings with cortisol levels assessed using additional sampling techniques.

The third and final finding from the current study flows from the univariate moderation models, which revealed that at greater levels of co-twin relationship quality, the proportion of variance in urinary cortisol explained by genetic factors was greater. In contrast, the proportion of variance explained by nonshared environmental factors was greatest when relationship quality was low. These findings suggest that at greater levels of co-twin relationship quality, genetic factors become more influential, but when co-twin relationship quality is lower, such factors become less influential, and the impact of environmental factors is magnified. These findings were somewhat unexpected, as previous studies examining cG×Es have reported significant interactions between deleterious environments and measured genetic variants, providing evidence of diathesis-stress (Monroe and Simons, 1991). The findings from the current study, however, seem to be more in line with previous studies reporting greater genetic expression in less restrictive environments (Dick et al., 2007, 2001), as well as Bronfenbrenner and Ceci's (1994) bioecological model. In this way, a closer connection with one's co-twin appears to operate as a less restrictive or enriched environment, enhancing underlying genetic factors. Alternatively, a more strained relationship with one's co-twin appears to represent a more restrictive or adverse set of environmental influences, which may diminish genetic influences on a given phenotype (Burt and Klump, 2014; Raine, 2002).

This finding has important implications for future research examining processes related to interpersonal relationships and HPA axis activity and indicates that greater contact with others may not *directly* regulate HPA axis activity, but may still be implicated through G×Es. While previous studies have noted the independent influence of genetic and environmental factors on cortisol levels (Bartels et al., 2003b; Inglis et al., 1999; Riese et al., 2009; Rietschel et al., 2017; Tucker-Drob et al., 2017), the findings from the current study indicate that the proportion of variance in cortisol levels explained by both sets of factors varies across social context. These findings align with previous studies employing both latent and measured gene approaches (Gerritsen et al., 2017; Mueller et al., 2011; Ouellet-Morin et al., 2008; Tucker-Drob et al., 2017; Tyrka et al., 2009), but the majority of these studies examine deleterious environments as moderators such as childhood adversity (Ouellet-Morin et al., 2008; Tyrka et al., 2009). The findings from the current study indicate that positive (or enriching) environments may be just as impactful in moderating genetic and environmental factors. Importantly, these findings align with previous studies reporting significant associations between exposure to positive interpersonal relationships and HPA axis activity (Adam and Gunnar, 2001; Papp et al., 2009; Pendry and Adam, 2007; Powers et al., 2006; Smyth et al., 2015), including those reporting associations between such relationships and adrenocortical attunement (Ha et al., 2016; Rankin et al., 2018). The continued examination of environmental context on HPA axis activity, and

attunement-based processes in particular, would benefit future research, as it appears that such factors contribute both directly and interactively with underlying genetic and additional environmental factors involved in generating variability in cortisol levels.

Alongside these contributions, the results of the current study should be interpreted with caution due to a number of limitations. While the results of the primary analysis were replicated using bootstrapping procedures that limit bias stemming from reduced power (Fu et al., 2005; Mooney and Duval, 1993), the employed sample size was modest and power was limited. Future research examining similar research questions with better powered samples would provide additional insight. Additionally, the employed urinary cortisol measure reflects cortisol concentrations across a 12-hour period. While this provides a more comprehensive measure of cortisol concentrations than measures more focused on momentary fluctuations, future studies employing a measure capturing a longer timeframe would be beneficial, with hair-based measures serving as a prime candidate for future studies (Russell et al., 2015). Related, the current study only focused on cortisol (specifically urinary cortisol), a product of the HPA axis. While a significant amount of research has focused on examining genetic and environmental factors that explain variability in cortisol levels, studies have identified biomarkers tapping other physiological systems, including the autonomic nervous system, and its constituent branches—the sympathetic and parasympathetic nervous systems (Davis and Granger, 2009; Gordis et al., 2010). Future research would benefit from a closer examination of the role of gene-environment interplay in the functioning of other, alternative systems.

The current study focused on twin dyads and previous research has indicated that twins possess a unique relationship (Fraley and Tancredy, 2012; Neyer, 2002; Tancredy and Fraley, 2006). While previous studies have found that twin-based samples do not systematically differ from samples comprised of singletons (Barnes and Boutwell, 2013), such findings do not necessarily extend to *relationships* between co-twins (Lahey and D'Onofrio, 2010). Future research would benefit from examining whether other interpersonal relationships (e.g., parents, peers, romantic partners) moderate genetic and environmental factors involved in cortisol output. Additionally, like any other analytic model, twin-based research designs are subject to strict assumptions and violation of these assumptions may result in biased estimates (Barnes et al., 2014). While the results of a recent simulation study indicated that estimates from twin-based research designs are largely robust to such violations (Barnes et al., 2014), the consideration of these assumptions in future research remains critical. Also in line with these considerations, it is worth noting that the moderator examined in the current study (i.e., co-twin relationship quality) is the result of a combination of genetic and environmental factors. While the models estimated in both the primary and supplemental analyses address genetic factors that covary between relationship quality and urinary cortisol levels, it is possible (and even likely) that additional genetic factors related to relationship quality persist. In light of this possibility, the use of the term "gene-environment interaction" may be somewhat inappropriate in this context, as it is possible that moderating influences may be comprised of both genetic and environmental factors. Future research would benefit from the continued development of analytic strategies aimed at addressing this possibility more directly.

The current study examined the extent to which co-twin relationship quality moderates genetic and environmental factors that contribute to individual differences in urinary cortisol levels. The results indicated that at greater levels of co-twin relationship quality, heritability estimates of urinary cortisol were also greater, while environmental factors were lower. These findings shed light on factors contributing to individual differences in HPA axis activity, as social context, including the quality of interpersonal relationships, may moderate underlying genetic and environmental factors. Additionally, these findings provide support for a bioecological model in which more enriching, or less restrictive, environments may promote genetic factors, but deleterious environments may overcome heritability and emphasizing environments. These findings demonstrate the importance of continued biosocial integration in this area of inquiry, as such efforts have the ability to provide a fuller and more precise understanding of behavior as well as the physical and mental health problems that stem from chronic cortisol secretion.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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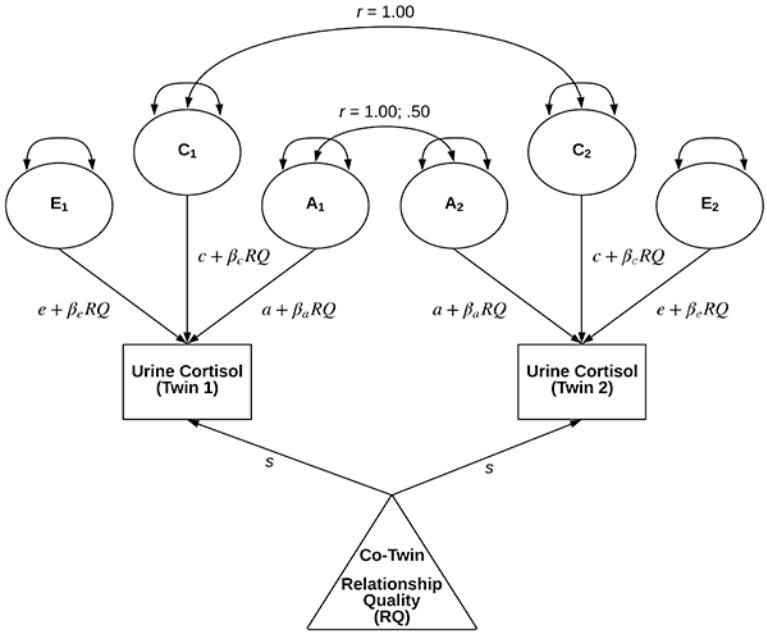


**Figure 1.**

Univariate Moderation Model.

Note: Co-twin relationship quality is presented as *RQ* and was regressed on the urinary cortisol measures for Twin 1 and Twin 2 (represented as path *s*) prior to estimating moderating effects. The residual variance of the urinary cortisol measure was partitioned to estimate additive genetic (A), shared environmental (C), and nonshared environmental (E) influences. The included multiplicative interaction terms ($\beta_a RQ$, $\beta_c RQ$, and $\beta_e RQ$) provide an estimate of the extent to which A, C, and E are moderated by varying levels of co-twin relationship quality (*RQ*). The net effect of each latent parameter combined with co-twin contact is represented as the sum of the direct effect (*a*, *c*, and *e*) and the respective interaction term. All models included controls for age (measured continuously in years), sex (0 = female; 1 = male), race (0 = Caucasian; 1 = all other races) and economic adversity (1 = more money than needed; 2 = just enough money to meet needs; 3 = not enough money to meet needs).

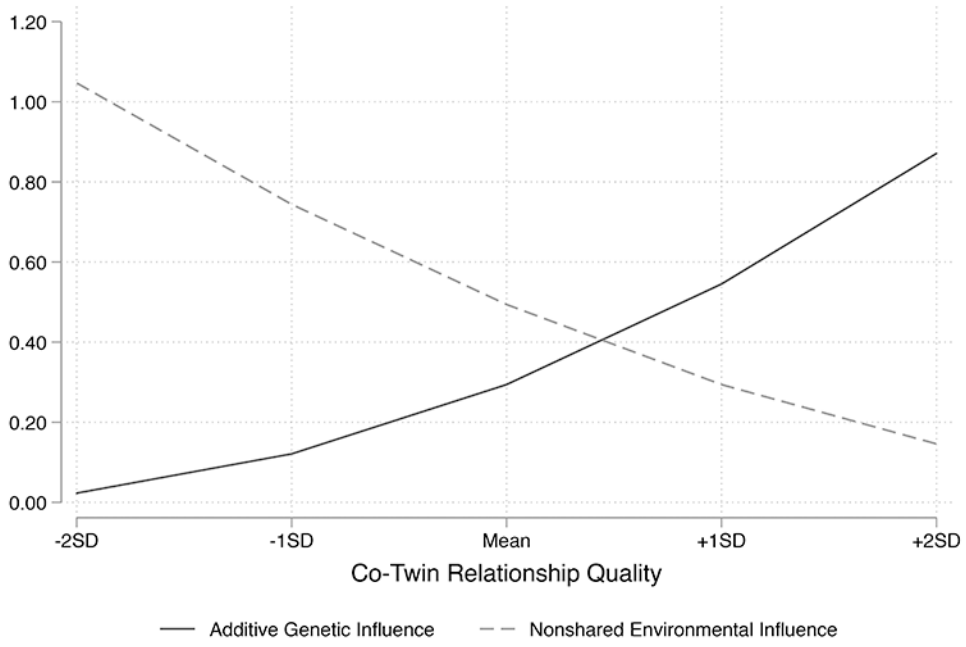


**Figure 2.**

Results from Extended Univariate Moderation Model.

Note: The plotted coefficients reflect unstandardized variance estimates in urinary cortisol explained across levels of co-twin relationship quality. The urinary cortisol measure was winsorized to three standard deviations of each participant's mean and log-transformed. The urinary cortisol and co-twin relationship measures were *z*-transformed (mean = 0; SD = 1). Any covariance between the urinary cortisol and co-twin contact measures was residualized prior to the estimation of the models. All models included controls for age (measured continuously in years), sex (0 = female; 1 = male), race (0 = Caucasian; 1 = all other races) and economic adversity (1 = more money than needed; 2 = just enough money to meet needs; 3 = not enough money to meet needs).

**Table 1.**

Descriptive Statistics of Study Measures.

| | Full Sample | | | MZ Twins | | | DZ Twins | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD/n** | **Min-Max** | **Mean** | **SD/n** | **Min-Max** | **Mean** | **SD/n** | **Min-Max** |
| Urinary Cortisol mg/g (mean) | 2.62 | .67 | .74 – 4.38 | 2.56 | .69 | .74 – 3.97 | 2.69 | .64 | .92 – 4.38 |
| Co-Twin Relationship Quality (mean) | 13.99 | 2.24 | 4 - 17 | 14.46 | 2.03 | 8 - 17 | 13.49 | 2.36 | 4 - 17 |
| Age (mean) | 52.52 | 11.46 | 34 - 82 | 53.10 | 11.19 | 34 - 82 | 51.88 | 11.76 | 35 - 81 |
| Sex (%) | | | 0 - 1 | | | 0 - 1 | | | 0 - 1 |
| Male | 38.26% | 114 | | 43.59% | 68 | | 32.39% | 46 | |
| Female | 61.74% | 184 | | 56.41% | 88 | | 67.61% | 96 | |
| Race (%) | | | 0 - 1 | | | 0 - 1 | | | 0 - 1 |
| Caucasian | 94.30% | 281 | | 96.15% | 150 | | 92.25% | 131 | |
| All Other Races | 5.70% | 17 | | 3.85% | 6 | | 7.75% | 11 | |
| Money to Meet Needs (%) | | | 1 - 3 | | | 1 - 3 | | | 1 - 3 |
| More than Enough | 27.70% | 82 | | 28.39% | 44 | | 26.95% | 38 | |
| Just Enough | 52.70% | 156 | | 59.35% | 92 | | 45.39% | 64 | |
| Not Enough | 19.59% | 58 | | 12.26% | 19 | | 27.66% | 39 | |
| N | | 298 | | | 156 | | | 142 | |

Abbreviations: *SD* = standard deviation; *Min* = minimum value; *Max* = maximum value.

Note: Urinary cortisol measure was winsorized to three standard deviations of each participant's mean and log-transformed.

**Table 2.**

Cross-Twin and Cross-Trait Correlations for the Full Sample and Twin Subsamples.

| | Full Sample | | Monozygotic Twins | | Dizygotic Twins | |
|---|---|---|---|---|---|---|
| | ***r*** | ***p*-value** | ***r*** | ***p*-value** | ***r*** | ***p*-value** |
| **Cross-Trait Correlation** | | | | | | |
| Urinary Cortisol and Co-Twin Relationship Quality | .08 | .19 | **.19** | **.02** | .01 | .88 |
| **Cross-Twin Correlation** | | | | | | |
| Co-Twin Relationship Quality | **.59** | ***p* < .001** | **.66** | ***p* < .001** | **.51** | ***p* < .001** |
| Urinary Cortisol µg/g | **.25** | **.001** | **.39** | ***p* < .001** | **.19** | ***p* < .001** |

Note: Results presented are Pearson zero-order correlation coefficients. Prior to estimation of correlation coefficients, the urinary cortisol measure was winsorized to three standard deviations of each participant's mean and log-transformed. Bolded correlation coefficients have an accompanying *p*-value that is less than .05.

**Table 3.**

Results of Univariate Biometric Models and Univariate Moderation Models

| | A | $\beta_A$ | C | E | $\beta_E$ | $\chi^2$(df) | −2LL(np) | Δ−2LL(np) | CFI | TLI | RMSEA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Univariate Models** | | | | | | | | | | | |
| Baseline Model | .59** | -- | .00 | .70** | -- | 19.29(27) | −383.30(7) | -- | 1.00 | 1.00 | .00 |
| | (.08) | -- | (.00) | (.06) | -- | | | | | | |
| Best-Fitting Model | .59** | -- | -- | .70** | -- | 19.97(28) | −383.30(6) | .00(1) | 1.00 | 1.00 | .00 |
| | (.08) | -- | -- | (.06) | -- | | | | | | |
| **Model with Interactions** | | | | | | | | | | | |
| Full Model | .54** | .20* | -- | .70** | −.16** | -- | −339.99(9) | -- | -- | -- | -- |
| | (.08) | (.09) | -- | (.05) | (.04) | | | | | | |

Abbreviations: *df* = degrees of freedom; *−2LL* = −2 loglikelihood; *np* = number of free parameters; *CFI* = comparative fit index; *TLI* = Tucker-Lewis Index; *RMSEA* = root mean square error of approximation.

Note: Robust standard errors presented in parentheses. The urinary cortisol measure was winsorized to three standard deviations of each participant's mean and log-transformed. The urinary cortisol and co-twin contact measure were *z*-transformed (mean = 0; SD = 1). Any covariance between the urinary cortisol and co-twin contact measures was residualized prior to the estimation of the models that include interaction terms. All models included controls for age (measured continuously in years), sex (0 = female; 1 = male), race (0 = Caucasian; 1 = all other races), and economic adversity (1 = more money than needed; 2 = just enough money to meet needs; 3 = not enough money to meet needs). Change in −2 loglikelihood assessed using likelihood ratio tests. Some model fit indices are not available for models involving an interaction term.

** $p < .001$

* $p < .05$