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Chronic stress exposure, diurnal cortisol slope, and implications for mood and fatigue: Moderation by multilocus HPA-Axis genetic variation

Lisa R. Starr[a,*], Kimberly Dienes[b], Y. Irina Li[a], Zoey A. Shaw[a]

[a] University of Rochester, United States
[b] University of Manchester, United Kingdom

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ABSTRACT

Chronic stress exposure has been shown to alter hypothalamic-pituitary-adrenal (HPA) axis functioning, which may mediate its effects on psychopathology and negative health outcomes. The nature of the chronic stress-HPA axis dysregulation is unclear and individuals likely vary in the extent to and manner in which indices of HPA axis regulation, such as diurnal cortisol slope, are influenced by chronic stress. We examined whether HPA-axis-linked genetic variation moderates the association between chronic stress and diurnal cortisol slope, and potential implications for mood and fatigue (possible manifestations of negative clinical outcomes). 211 adolescents (M age 15.89, 54.5% female) completed chronic stress interviews and provided DNA samples. Participants then provided saliva samples at waking and 12 h post-waking for two consecutive weekdays. HPA-axis genetic variation was calculated using a multilocus genetic profile score (MGPS) approach, using ten SNPs from CRHR1, NR3C1, NR3C2, and FKBP5 to generate an additive score of HPA-axis-linked genetic risk. Neither chronic stress nor MGPS directly predicted diurnal slope, but MGPS moderated the association between chronic stress and diurnal slope, with stress predicting a high waking cortisol followed by steep slope among youth with low but not high MGPS scores. MGPS also interacted with chronic stress to predict both negative affect and fatigue, and moderated the indirect effect of chronic stress on mood and fatigue via diurnal slope. Results suggest that diurnal cortisol regulation may be one mechanism by which genetic risk intensifies the association between chronic stress and negative outcomes.

1. Introduction

1.1. Overview

Some are more likely than others to succumb to health problems following stress exposure. Genetic risk likely contributes to this variability, but much is unknown about specific physiological mechanisms bridging genetic variation to health-related phenotypes. Here, we explore an important potential factor, diurnal cortisol slope, in relation to chronic stress and multilocus HPA-axis genetic variation.

1.2. Diurnal cortisol slope

Cortisol, a primary product of the hypothalamic-pituitary-adrenal (HPA) axis, is secreted both in response to threat and in a strong, consistent pattern over the course of the day (diurnal cortisol rhythm). Cortisol has a multitude of effects on the body, including marshalling energy and shutting down non-essential systems to cope with threat, and also impacts memory and mood (Chrousos and Gold, 1992). Therefore, dysregulated cortisol secretion can lead to numerous negative physiological, cognitive and emotional outcomes, and has been directly implicated in mood disruptions and fatigue (e.g., Powell et al., 2013; van Eck et al., 1996).

Cortisol levels are typically elevated upon wakening, peak 30–40 min later (cortisol awakening response [CAR]), and then slowly decline over the course of the day, reaching their nadir around bedtime (Adam and Kumari, 2009). Individual differences in diurnal cortisol slope (the rate of daily decline) are tied to negative mental and physical health conditions, with flatter slope predicting same-day negative affect (NA), fatigue, depression, and other critical outcomes (Adam et al., 2006, 2017). Therefore, better understanding the source of individual differences in diurnal cortisol slope is relevant to the etiology of numerous mental and physical health problems.

* Corresponding author.
E-mail address: lisa.starr@rochester.edu (L.R. Starr).

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1.3. Chronic stress and diurnal slope

Chronic stress (ongoing environmental strains) is a key predictor of NA, fatigue, major depression, and other health outcomes (e.g., Hammen, 2005; McEwen, 2004), conditions also linked to flattened slope. It is feasible that alterations in diurnal slope play a mechanistic role linking chronic stress to diverse outcomes. Research on chronic stress and diurnal cortisol slope is mixed, with chronic stress linked in some studies to *flattened* (dysregulated) slope (Adam et al., 2017) and in others to *steeper* slope (Ockenfels et al., 1995; Steptoe et al., 2000). These discrepant findings may imply existence of important moderators. Miller et al., 2007argued that repeated HPA-axis activation alters the relationship between chronic stress and cortisol secretion. Initially, stress leads to elevations in diurnal cortisol, facilitating recruitment of energy and coping resources. However, repeated or sustained cortisol elevations may lead to a breakdown in the negative feedback system of cortisol secretion, resulting in flattened slopes and corresponding negative clinical and health outcomes. Heterogeneity in the relationship of chronic stress to diurnal slope suggests some individuals are more prone than others to a breakdown in the healthy relationship between stress and cortisol secretion. One possible factor may be genetic risk (Miller et al., 2007).

1.4. Genetic contributors

Genetic variation has been highlighted as an important moderator of associations between stress and negative outcomes. A substantial body of research documents gene-environment interactions (G × E) in predicting psychopathological outcomes, including for genes directly related to HPA-axis functioning like CRHR1 and FKBP5 (e.g., Starr et al., 2014). Likewise, HPA-axis linked genetic variants predict indices of HPA-axis regulation in response to induced challenges, including neuroendocrine response to laboratory-induced stressors and dexamethasone challenges (e.g., DeRijk et al., 2006; Menke et al., 2013). Although less research has explored genetic moderation of diurnal cortisol rhythms collected in naturalistic settings, genome-wide association studies (GWAS), GWAS-based polygenic risk score analyses, and behavioral genetic evidence suggest diurnal cortisol patterns are influenced by genetic factors (Utge et al., 2018; Van Hulle et al., 2012; Velders et al., 2011). Further, Cicchetti et al. (2011) showed a CRHR1 haplotype interacted with maltreatment exposure to predict flatter slope.

Most previous research has explored individual single nucleotide polymorphisms (SNPs) or haplotypes. Reliability of single-variant candidate gene designs has recently come under fire (Dick et al., 2015); although this issue remains under debate, many argue individual variant G × E effects are likely very small. To amplify power, several research groups have devised multilocus genetic profile scores (MGPSs), reflecting additive presence of multiple risk alleles within a biological system (Nikolova et al., 2011; Vrshek-Schallhorn et al., 2015). Use of MGPSs results in substantially higher effect sizes compared to single-SNP designs (e.g., Nikolova et al., 2011; Starr and Huang, in press). Pagliaccio et al. (2014) recently developed a MGPS using 10 HPA-axis-related SNPs to capture stress-system genetic risk, which predicted children's increased cortisol following a laboratory stressor and interacted with early life stress to predict decreased hippocampal and amygdala volume (an effect mediated by increased cortisol secretion) and amygdala connectivity (Pagliaccio et al., 2014, 2015). HPA-axis MGPSs also appear to robustly moderate associations between naturalistic stress exposure and affective outcomes (Feurer et al., 2017; Starr and Huang, in press). Collectively, these findings support the MGPS approach and implications of HPA-axis genetic variation for health outcomes.

However, no research has examined associations between HPA-axis multilocus genetic risk and diurnal cortisol rhythms. If HPA-axis genetic variation predicts differential associations between chronic stress and diurnal slope, it may explain variations in vulnerability to negative outcomes related to chronic stress. For example, daily fatigue and NA are linked to stress exposure and represent daily manifestations of physical and mental health conditions related to diurnal slope (e.g., depression, chronic fatigue); HPA-axis genetic variation may predict stronger associations between chronic stress and these outcomes via flatter diurnal slope. These ideas are especially important to examine during adolescence, a period of rapid changes in adrenocortical functioning and stress reactivity (Gunnar et al., 2009; Schreiber et al., 2006; Shirtcliff et al., 2012).

1.5. Current study

We examined whether an HPA-axis MGPS (Pagliaccio et al., 2014) moderates the link between chronic stress and diurnal cortisol slope among adolescents. In supplemental hypotheses, we examined MGPS in relation to fatigue and NA; we predicted that a) genetic risk would intensify associations between chronic stress and both fatigue and NA, and b) using a moderated mediation framework, diurnal slope would differentially account for associations between chronic stress and fatigue/NA as a function of genetic risk.

2. Materials and methods

2.1. Participants

The full sample included 241 adolescents aged 14–17 years, of whom 211 returned labelled cortisol samples (see section 2.5.1; M_{age} = 15.89, SD = 1.10; 54.5% female). Participants were recruited from the community of a mid-sized metropolitan area using a variety of methods including advertisements posted in the community and online, a targeted commercial mailing list, and ResearchMatch, a national research health volunteer registry. Participants identified the following racial/ethnic backgrounds: 75.4% White, 11.4% Black, 4.3% Asian, 3.8% multiracial, 4.7% other or no race reported, and 0.5% Native American; 10.0% identified as Hispanic or Latino. For further recruitment and demographic details, see Starr et al. (2017). Other publications using this sample have examined non-overlapping hypotheses (Starr et al., 2017; Starr and Huang, in press).

Exclusion criteria included prior diagnosis of bipolar or psychotic disorder, any major physical, neurological, or pervasive developmental disorder, English reading or language difficulties, and prior participation of another household member in the study. Additional cortisol study component exclusion criteria included use of steroid-based medications, pregnancy, and neuroendocrine disorder presence.

2.2. Measures

2.2.1. Chronic stress

The Chronic Stress Interview (CSI), a portion of the UCLA Life Stress Interview (Hammen et al., 1987), is a semi-structured interview adapted for use with late adolescents (Hammen and Brennan, 2001). The CSI evaluates the nature and quality of ongoing conditions in the last six months across multiple domains of the adolescent's life: close friendships, peer relations, family life, romantic life, academics, and behavioral issues. Interviewers underwent a rigorous training protocol and attained required reliability benchmarks. Interviewers rated the youth's ongoing stress based on objective features of individual domains on a five-point scale, with behavioral anchors ranging from 1 (exceptionally good conditions) to 5 (extreme adversity). Scores across all domains were averaged. Interrater reliability was good (intraclass correlation = .87).

2.2.2. Negative affect and fatigue

The negative affect scales of the Profile of Mood States-15 (POMS-15; Cranford et al., 2006) includes 12 items assessing anxious mood,

depressed mood, anger, and fatigue. Each item is rated on a five-point Likert Scale. All items were averaged for an overall score of NA, and the three-item fatigue subscale utilized to assess fatigue. Scores were averaged across the six assessment points (see Section 2.5). Internal reliability was computed at each sampling point and averaged; mean Cronbach's alphas were 0.83 for NA and 0.84 for fatigue.

2.2.3. Pubertal development

The Pubertal Development Scale (PDS; Petersen et al., 1988) is a widely-used self-report scale assessing physical maturation. The five PDS items were averaged for an overall score.

2.2.4. Depression

Adolescent's diagnosis of major depressive disorder (MDD) was assessed using the Schedule for Affective Disorders and Schizophrenia for School-Aged Children–Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997), a semi-structured diagnostic interview with strong validity and reliability, administered by graduate students and a bachelor's level research assistant following intensive training and reliability attainment. Current MDD diagnosis was used as a covariate in the present analyses. Interrater reliability was perfect (kappa = 1.0).

2.3. Genotyping and MGPS computation

2.3.1. Genotyping

Saliva samples were obtained from adolescents using Oragene™ (DNA Genotek, Ontario, Canada) collection kits. DNA was submitted to the University of Wisconsin-Madison Biotechnology Center for analysis. DNA concentration was detected and quantitated using the Quant-iT™ PicoGreen® dsDNA kit (Life Technologies, Grand Island, NY) and extracted using standard salting-out procedure. Genotyping was completed using KBiosciences competitive allele specific PCR SNP genotyping assay based on dual FRET (KASPar). KASPar assays were amplified with the Eppendorf Mastercycler pro384 thermal cycler using allele specific primers. End point fluorescence signal was analyzed using the Synergy 2 (BioTek®) plate reader and Gen5™ software program.

In accordance with Pagliaccio et al.'s (2014) HPA axis MGPS, genotype data were obtained for risk alleles of 10 SNPs from four HPA axis-related genes: *CRHR1* (rs4792887 *T* allele, rs110402 *G* allele, rs242941 *T* allele, rs242939 *G* allele, rs1876828 *G* allele), *NR3C1* (rs41423247 *G* allele, rs10482605 *T* allele, rs10052957 *A* allele), *NR3C2* (rs5522 *G* allele), and *FKB5* (rs1360780 *T* allele). SNPs were chosen for associations with elevated cortisol, depression, and/or related phenotypes and were pruned to reduce linkage disequilibrium; extensive detail on the development and coding scheme of this MGPS is available elsewhere (Pagliaccio et al., 2014)

Distributions of genotypes for individual SNPs are available upon request. All distributions were in Hardy-Weinberg equilibrium ($\chi^2(1) \leq 2.82$, $ps > .05$) with the exception of rs1876828 $\chi^2(1) = 4.12$, $p = .041$. Re-running analyses excluding this SNP did not impact results.

2.3.2. Computation of MGPS

HPA axis genetic profile scores were computed following procedures of Pagliaccio et al. (2014). SNPs were coded for presence (1) or absence (0) of at-risk genotypes (.5 codes were assigned to heterozygotes in cases of allelic rather than genotypic effects). Individual SNP codes were summed, with higher MGPS reflecting higher genetic risk (possible range 0–10, sample range 2–9. We permitted up to two missing genotypes (20%) per person, rescaling using available SNPs when necessary.

2.4. Procedure

Participating youth and parents provided consent/assent, after which they were separately interviewed, completed a battery of questionnaires, and provided DNA samples. Families received $160 for participation in study procedures and were entered into raffles. The Research Subjects Review Board of the University of Rochester approved study procedures.

2.5. Salivary cortisol procedures

At the end of their laboratory visit, participants were given materials to collect salivary cortisol from their home. Families were given detailed verbal and written instructions on how to collect ambulatory saliva samples and were provided with a website with additional instructions and videos demonstrating procedures. Study staff and instructional materials emphasized the importance of accurate timing and reporting. Participants were instructed to collect ambulatory salivary cortisol samples four times a day for 2 consecutive days. Sample collection days were timed between Tuesday and Thursday because of findings suggesting substantial differences in morning cortisol on Mondays and on Fridays/ weekends (e.g., Schlotz et al., 2004). Participants collected samples immediately after waking (Sample 1: S1), 30 min after waking (Sample 2: S2), 60 min after waking (Sample 3: S3), and 12 h after waking (Sample 4: S4) on 2 consecutive weekdays (second and third samples of the day were not used in current analyses). At S2, S3, and S4, participants completed mood measures. Because toothpaste and certain food and drink can degrade or dilute salivary cortisol, adolescents were asked to refrain from brushing teeth, eating, and drinking for 30 min prior to collecting samples (Kudielka et al., 2007). Samples were collected using Salivate® Cortisol (Sarstedt Inc.) synthetic swabs designed for determination of cortisol from saliva. To collect each sample, participants placed a swab in their mouth and let it collect saliva until saturated. Participants indicated whether they ate, drank, brushed their teeth, or participated in vigorous activity in the 30 min before each sample. Participants also recorded their waking time, how many hours they slept, their tobacco use, alcohol, caffeine, and high sugar food consumption, and exercise. Female participants provided information on their menstrual cycle.

Completed samples and information forms were mailed to the lab, where samples were stored at –20 °C. Of the original sample of 241, 12 were excluded from cortisol procedures for medical reasons, and 18 declined to participate in cortisol procedures or failed to return samples, leaving 211 participants that were assayed. Samples were shipped to Dresden, Germany, where they were assayed for cortisol using time-resolved immunoassay with fluorescence detection (dissociation-enhanced lanthanide fluorescence immunoassay; Dressendörfer et al., 1992). The laboratory conducting the assays reported intra- and inter-assay coefficients of variance below 12%.

Electronic MEMS® caps recorded the time and date that each bottle containing Salivettes was opened for a randomly selected 28 of the 211 participants (13.3%) to check accurate time reporting. Data were downloaded using MEMS software (PowerView, Version 3.5.2). Of the 224 data points checked using MEMS, 6 were missing values, leaving a total of 218 data points (we report on accuracy on all data points to reflect overall compliance). The timing and sample intervals participants reported closely corresponded to MEMS data. For the critical interval between S1 (awakening) and S2 (30 min post-awakening), MEMS-recorded time intervals deviated from self-reported time intervals by an average of only 2.63 min (average MEMS-recorded interval = 31.94 min), and 96% of MEMS-based intervals were within seven minutes of the self-reported interval. Similar accuracy was found for the S2 to S3 interval. When examining all data points recorded using MEMS, 83% were within 10 min of the reported sample time, mainly due to some larger differences between MEMS time and S4 reporting (sampling accuracy is less important for S4 than for CAR).

2.5.1. Cortisol data cleaning and slope calculations

Diurnal cortisol slope is distinct from CAR (S1, S2 and S3) and therefore calculated using the difference from S1 (awakening) to S4

(12 h past waking) divided by the time in hours. This technique for measurement of diurnal slope is representative of the literature on diurnal slope according to systematic review and meta-analysis (Adam et al., 2017). Indeed, diurnal slope calculated across two points over two days has been shown to correlate 0.94 with cortisol slopes based on 6–7 samples per day over 2 days (Adam and Kumari, 2009). However, two days is not enough to capture within-person variability, so outcomes were averaged across the two days.

Sampling time is crucial for CAR and waking sampling, but not as essential for end-of-day sampling. Frequently end-of-day sample is taken right before bedtime, which is variable, therefore, as long as the last sample of the day was in the evening and recorded accurately according to self-report and MEMS, the day of sampling was retained. From the original 211 participants with cortisol data, 196 were retained for diurnal slope analyses (92.5%). Three participants returned cortisol with consistently anomalous values (< 1 or greater than 300 nmol/ml), one participant returned only S2 or S3, one participant had no reported sampling times, two participants were missing S4 on both days, and eight participants returned only one day of sampling and the other day had extreme errors in sampling compliance or anomalous cortisol values for Sample 1 or 4 (< 1 nmol/l). Seven additional participants only returned one sampling day, but appeared to follow sampling protocol for the second day of sampling so were retained for analyses. S1 analyses were conducted with 201 participants (5 participants with S1 but not S4). Cortisol values at each sampling time were winsorized to correct for extreme outliers (> 3 SDs). There were no differences between the cortisol sample and remainder of the sample on age, gender, or MDD, but participants in the cortisol sample were more likely to be White ($p < .05$).

2.6. Analytical approach

All interactions and conditional process analyses were conducted using PROCESS macros (Hayes, 2013) in SPSS 25.0. MGPS and chronic stress were mean-centered and entered as main effects and in interactions. Significant interactions were probed using simple slope tests conducted at one SD above and below the mean. We also conducted Johnson-Neyman technique (JNT) region of significance tests to identify levels of MGPS at which chronic stress significantly predicted the outcome. To correct for multiple tests, we applied False Discovery Rate (FDR) adjustments (Benjamini and Hochberg, 1995) to all G × E p values, with FDR designated at .05.

Sex, race, and pubertal maturation were included as covariates in all G × E models. Additional covariates were included if they showed bivariate correlations with outcome variables (diurnal slope, S1, S4) at $p \leq .10$ (the inclusive alpha level was selected as a conservative measure). Specific biobehavioral variables examined included day-of-sampling behaviors (vigorous exercise, consumption of tobacco products, alcohol, or high-sugar foods, whether it was a school day), day-of-sampling sleep variables (wake time, total sleep time), compliance with sampling procedures (eating, drinking or engaging in vigorous exercise within 30 min before sampling), and health variables (follicular stage [boys coded zero], use of hormonal contraceptives, and MDD). To account for unmodeled nonlinearity in G × E models (Dick et al., 2015), we ran additional models in which quadratic effects for chronic stress and MGPS were included as covariates.

Conditional process analysis (Section 3.5) was used to examine whether diurnal slope differentially mediated associations between chronic stress and fatigue/NA as a function of MGPS. Using PROCESS, chronic stress was entered as the independent variable, diurnal slope as the mediator, and MGPS modifying the indirect path via moderation of the a path (association between chronic stress and diurnal slope). Fig. 1 illustrates this conceptual model. Covariates (see Section 3.1) were included in the a path model, and bootstrapping (5000 resamples) was used to estimate asymmetrical, bias-corrected 95% confidence intervals for the indirect effect at each level of the moderator, and for the index of moderated mediation.

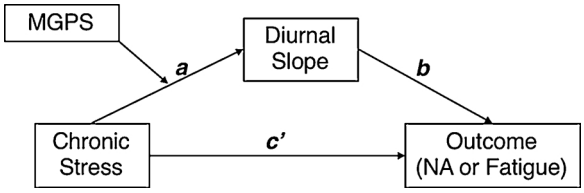

Fig. 1. Conceptual Model for diurnal slope as a moderated mediator of the association between chronic stress and NA/ fatigue, as a function of MGPS genetic risk.

When interpreting results for diurnal slope, note that slope values are generally negative, lower values (i.e., higher absolute values with a negative sign) constitute steeper slope, and higher values (i.e., closer to zero) reflect flatter slope. Therefore, when considering associations with predictor variables, positive effect sizes indicate prediction of flatter slope.

3. Results

3.1. Biobehavioral and demographic covariates

As a first step, we examined correlations between health, behavioral, and demographic variables and diurnal slope, waking cortisol (S1) and 12-hours post-waking cortisol (S4). Significant biobehavioral covariates at the inclusive .10 alpha level included a) diurnal slope: school day ($r = .17$) and mean wake time ($r = -.12$), b) S1: follicular stage ($r = .12$), and c) S4: school day ($r = .25$), mean wake time ($r = -.29$), mean hours slept ($r = -.21$), and MDD ($r = .19$). Results were retained when all covariates were included in cortisol models, and when no covariates were included.

3.2. Bivariate correlations and main effects

There were no significant main effects of either chronic stress or MGPS on diurnal slope, nor evidence of gene-environment correlation between MGPS and chronic stress (Table 1). Chronic stress (but not MGPS) predicted higher cortisol at S1 (marginally, $p = .075$) and S4 ($p = .038$). Diurnal slope was also correlated with NA and fatigue.

3.3. MGPS × chronic stress predicting diurnal cortisol

MGPS significantly moderated the relationship between chronic stress and diurnal slope (Table 2; interaction nominal $p = .008$, FDR-corrected $p = .013$). At M-1 SD MGPS, chronic stress predicted steeper slopes ($b = -.27$, $SE = .10$, $p = .006$), whereas at $M+1$ SD MGPS, chronic stress was not associated with slope ($b = .13$, $SE = .10$, $p = .205$) (Fig. 2). JNT indicated regions of significance at MGPS ≤ 4.10 (48th percentile) for chronic stress predicting steeper slope and MGPS ≥ 7.40 (96th percentile) for predicting flatter slope (the latter finding should be interpreted with caution given the small proportion of the sample above this threshold). Results were retained when quadratic terms were included. As a more conservative approach to addressing population stratification, we re-ran analyses in the Caucasian portion of the sample; results were replicated (interaction $p = .034$). The MGPS-chronic stress interaction did not differ by sex or pubertal maturation (three-way interaction $ps > .05$).

To better probe the nature of this interaction, we conducted exploratory analyses examining S1 and S4 cortisol as outcomes of the MGPS × chronic stress interaction (adjusting covariate inclusion, see Section 3.2).[1] MGPS significantly moderated the association between

[1] We did not examine S2 or S3 as outcomes, as these reflect CAR, well-established as a dissociable phenomenon from diurnal slope (Wilhelm et al.,

Table 1
Bivariate Correlations among Major Study Variables.

	1.	2.	3.	4.	5.	6.	7.
Diurnal Cortisol Slope	–						
Waking Cortisol	−.90***	–					
Waking + 12 h Cortisol	.24**	.09	–				
MGPS	.06	−.08	.02	–			
Chronic Stress	−.08	.13	.15*	.04	–		
NA	.15*	−.10	.12	.06	.22**	–	
Fatigue	.18*	−.14	.04	−.04	.02	.76***	–
M	−.53	8.57	1.98	4.59	2.21	1.46	1.90
SD	.38	4.20	1.55	1.40	.41	.39	.77

* $p < .05$.
** $p < .01$.
*** $p < .001$.

Table 2
Regression Results for Multilocus Genetic Profile Score (MGPS), Chronic Stress, and Their Interaction Predicting Diurnal Cortisol Slope.

	b	*SE*	*p*	95% C.I.
Intercept	−.51	.12	> .001	[−.75, -.27]
MGPS	.03	.02	.161	[−.01, .07]
Chronic Stress	−.07	.07	.306	[−.20, .06]
MGPS × Chronic Stress	**.14***	**.05**	**.008**	**[.04, .25]**
Covariates				
Caucasian Race	−.07	.07	.348	[−.21, .07]
Pubertal Maturation	−.07*	.03	.047	[−.13, .00]
Sex	−.02	.07	.747	[−.15, .11]
School Day	.10	.07	.167	[−.04, .24]
Wake Time	−.01	.04	.831	[−.08, .06]

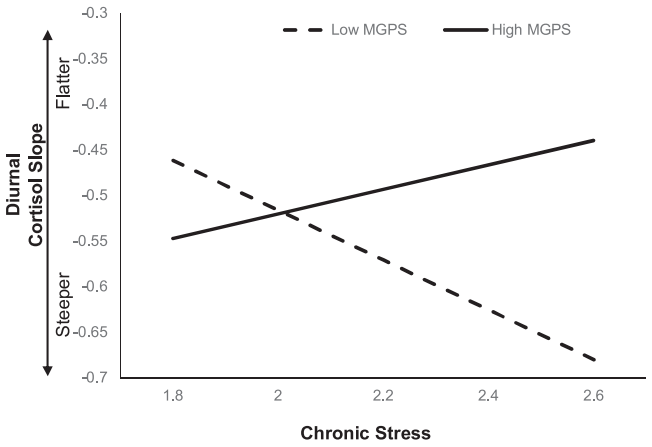

Fig. 2. Association between chronic stress and diurnal cortisol slope, at high and low multilocus genetic profile scores (MGPS).
Note. Estimates control for mean-centered covariates (race, sex, pubertal maturation, school day, wake time). High and low MGPS are defined as $M \pm 1$ *SD*.

chronic stress and S1 cortisol (interaction $b = -1.90$, $SE = .59$, nominal $p = .002$, FDR-adjusted $p = .008$, 95% C.I. [−3.22, −.81]). At *M*-1 *SD* MGPS, chronic stress strongly predicted higher waking cortisol ($b = 4.09$, $SE = 1.07$, $p < .001$), whereas at $M+1$ MGPS, chronic stress was unrelated to waking cortisol (b=-1.21, $SE = 1.15$, $p = .294$). JNT suggested chronic stress predicted S1 cortisol when MGPS ≤ 4.57 (54th percentile), and that at very high levels of genetic risk (MGPS ≥ 7.15, 95th percentile), chronic stress predicted *lower* waking cortisol (again, interpret latter result with caution given small portion of sample above this MGPS).

The interaction between MGPS and chronic stress predicting S4 cortisol was also significant (b= -.48, $SE = .21$, nominal $p = .024$, FDR-adjusted $p = .030$). Decomposition showed chronic stress predicted higher cortisol at *M*-1 SD MGPS ($b = .88$, $SE = .41$, $p = .034$), but not at $M+1$ SD MGPS (b=-.47, $SE = .42$, $p = .258$); JNT region of significance MGPS ≤ 3.53 (23rd percentile). Estimated diurnal patterns for high and low MGPS and chronic stress levels are presented in Fig. 3.

3.4. MGPS × chronic stress predicting mood and fatigue

MGPS significantly interacted with chronic stress to predict fatigue ($b = .29$, $SE = .11$, nominal p=.030, FDR-adjusted $p = .028$), with chronic stress predicting increased fatigue at $M+1$ SD MGPS ($b = .46$, $SE = .21$, $p = .030$) but not *M*-1 SD MGPS (b= −.20, $SE = .20$, $p = .305$). JNT revealed a region of significance of MGPS ≥ 5.47 (72nd percentile). MGPS also significantly moderated the association between chronic stress and NA ($b = .15$, $SE = .05$, nominal $p = .006$, FDR-adjusted $p = .013$); chronic stress predicted higher NA at $M+1$ SD MGPS ($b = .46$, $SE = 10$, $p < .001$), but not *M*-1 SD MGPS ($b = .04$, $SE = .10$, $p = .653$). JNT region of significance was MGPS ≥ 3.88 (23rd percentile). As the NA measure included fatigue items, we recalculated an NA variable excluding these items; results were retained ($p = .019$). Results were unaffected by inclusion of quadratic terms for MGPS and chronic stress.

3.5. Moderated mediation models

Fatigue was entered as the first outcome. In the *a* path (see Fig. 1), MGPS significantly moderated the association between chronic stress and diurnal slope (as reported earlier), and in the *b* path, diurnal slope predicted fatigue controlling for chronic stress ($b = .38$, $SE = .14$, $p = .010$). The direct effect of chronic stress on fatigue was non-significant ($b = .11$, $SE = .13$, $p = .399$). The indirect effect varied as a function of MGPS (moderated mediation index = .05, 95% CI [.01, .12]). At $M+1$ SD MGPS, the indirect effect was significant and negative (ab = −.10, CI [−.35, −.01]. This meets criteria for inconsistent mediation (MacKinnon et al., 2007), as the direct and indirect effects have opposite signs, indicating the mediator functions as a suppressor variable. That is, among those with low genetic risk, the presence of chronic stress predicts steeper diurnal slope that then predicts lower fatigue, thereby blunting the direct effect of chronic stress on fatigue and leading to a non-significant total effect. At $M+1$ SD MGPS, the indirect effect was non-significant and, notably, positive in sign ($ab = .05$, CI [−.01, .15]). Further analyses revealed that at very high levels of MGPS (90th percentile), the indirect effect was significant and positive ($ab = .08$, CI [.01, .21]), indicating that at very high genetic risk, chronic stress predicts flatter slope which in turn predicts higher fatigue; in other words, alterations in diurnal slope exposure leads to weaker relationships between chronic stress and fatigue at low MGPS, but stronger associations at high MGPS.

We next ran this model using NA as the outcome and found a similar pattern of results. The *a* path was as reported above. In the *b* path, flatter slope predicted higher NA ($p = .015$). There was significant moderated mediation (index = .03, 95% CI [.004, .06]), and again, the indirect effect was significant and negative at *M*-1 *SD* MGPS (ab= -.05, 95% CI [−.12, −.004]), the opposite sign from the positive direct effect ($b = .24$, $SE = .07$, $p < .001$), suggesting inconsistent mediation. In contrast, at $M+1$ *SD* MGPS, the indirect effect was positive in sign, although not significant, and at very high MGPS (e.g., 90th percentile), the indirect effect was positive and significant ($ab = .04$, 95th CI [.001, .10]).

(*footnote continued*)
2007). However, as a post-hoc exploratory analysis, we examined whether chronic stress interacted with MGPS to predict CAR, calculated using S1, S2, and S3 data as the area under the curve with respect to increase (AUC_i; see Starr et al., 2017 for calculation details).The interaction was non-significant, $p > .05$.

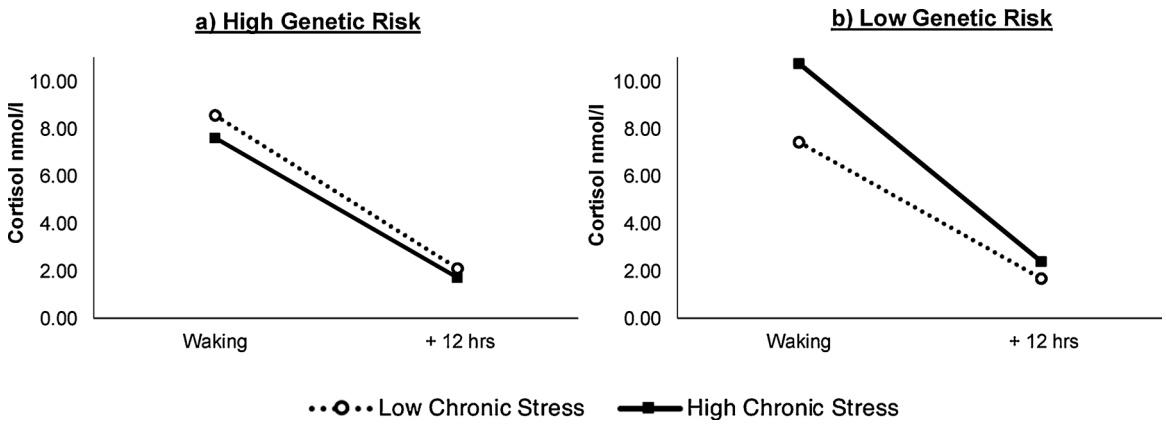

Fig. 3. Illustration of diurnal decline in salivary cortisol from waking to 12 h post-waking at by chronic stress at a) high and b) low HPA-axis multilocus genetic profile score (MGPS).
Note. Graphs depict regression function predicted values at $M \pm 1$ *SD* for chronic stress and MGPS, controlling for mean-centered covariates. Covariates included race, pubertal maturation, and sex, plus follicular stage for waking cortisol and school day, mean wake time, mean hours slept, and major depressive diagnosis for waking +12 cortisol (see Section 3.2).

4. Discussion

Results indicate HPA-axis-linked genetic variation may, in part, explain heterogeneity in associations between chronic stress exposure and diurnal cortisol slope variation. Specifically, youth at *low* genetic risk appear to adapt to chronic environmental strains over six months with a high waking cortisol, steeper diurnal decline, and higher end-of-day cortisol. The end-of-day result is somewhat at odds with other findings, as higher end-of-day cortisol contributes to *flatter* slope (Adam and Kumari, 2009). Thus, the MGPS-stress interaction predicting diurnal slope may be driven by adolescents with high chronic stress and low MGPS showing higher waking cortisol that largely recovers over the course of the day, although cortisol levels remain elevated for this group at 12 h post-awakening.

Elevated waking cortisol may provide needed resources to deal with the stress of the day, and elevated waking cortisol followed by steep diurnal slope is considered an adaptive response to ongoing stressors in the environment (Powell and Schlotz, 2012). This adaptive biological response to chronic stress may buffer adolescents at low HPA-axis genetic risk from negative effects of chronic stress, as previous findings indicated no association between chronic stress and depression for adolescents at low levels of this MGPS (Starr and Huang, in press). Further, we found youth with low MGPS scores showed no association between chronic stress and either fatigue or NA, compared to their high genetic risk peers. Steeper diurnal slope partially accounted for the protective effect of low MGPS on the relationship between chronic stress and fatigue/NA. That is, among those with low MGPS, chronic stress exposure may facilitate adaptive diurnal cortisol patterns, canceling out deleterious effects of chronic stress on these negative outcomes. Low MGPS is associated with lower cortisol reactivity to acute stressors (Pagliaccio et al., 2014), which may preserve negative feedback loops under conditions of chronic stress (Miller et al., 2007).

A different picture emerges for adolescents at elevated HPA-axis genetic risk, as chronic stress was unrelated to diurnal slope. This suggests a less environmentally contingent diurnal cortisol pattern likely reflecting HPA-axis dysregulation. Moreover, at the very highest levels of genetic risk (encompassing 4% of our sample), chronic stress was associated with the opposite diurnal cortisol pattern from low genetic risk—*lower* morning cortisol, followed by *flatter* diurnal slope. This dysregulated pattern may contribute to increased vulnerability to negative consequences of chronic stress, as adolescents at high genetic risk were at elevated risk of fatigue/NA as a function of chronic stress. This is consistent with studies suggesting these youth are more vulnerable than youth at low genetic risk to depression following chronic stress and other stressful contexts (Feurer et al., 2017; Starr and Huang, in press). Our moderated mediation results supported this model. In contrast to adolescents at low MGPS, for adolescents with moderately high MGPS, chronic stress was not associated with alterations in diurnal slope, such that these youth were not "protected" against effects of chronic stress on NA/fatigue (i.e., they lacked adaptive HPA axis functioning to counteract the impact of stress). Moreover, at the *very highest* levels of genetic risk, chronic stress predicted *flatter* slopes, contributing to *greater* NA/fatigue. This suggests alterations in diurnal cortisol slope as a function of chronic stress exposure are protective at low MGPS, but maladaptive for those at high MGPS.

Our findings suggest dysregulation in diurnal cortisol slope may represent one biological mechanism explaining the relation between HPA-axis multilocus genetic risk and negative outcomes (i.e., depression) under conditions of environmental stress (Feurer et al., 2017; Starr and Huang, in press). However, other mechanisms likely also contribute, as previous research indicated multilocus HPA axis genetic risk predicts elevated cortisol levels following an acute laboratory stressor in children (Pagliaccio et al., 2014). Further, multilocus HPA-axis genetic risk interacted with early life stress to predict increased amygdala volume, decreased hippocampal volume, and increased threat-related amygdala reactivity, with implications for problems like anxiety and emotion regulation (Di Iorio et al., 2017; Pagliaccio et al., 2014, 2015). Future research should incorporate other indices of HPA-axis functioning, such as latent trait cortisol (Stroud et al., 2016), reactivity to acute stressors, or dexamethasone challenge response, to capture other unique aspects of neuroendocrine functioning. Further, as the HPA-axis is implicated in biological processes like immune function, sleep, and memory (Glaser and Kiecolt-Glaser, 2005; Lupien et al., 2009), studies should examine other intermediate phenotypes linking HPA-axis genetic variation to risk for psychopathology and health problems.

Importantly, we examined the effects of *chronic* stress (strains sustained for at least six months) on diurnal stress, as moderated by genetic variation. Repeated or prolonged activation of the HPA axis due to presence of chronic stressors leads to break-down of the cortisol negative feedback system, resulting in hypocortisolism in response to stress (Miller et al., 2007). With initial stress onset, youth with high MGPS may show a pattern of hypercortisolism (consistent with studies linking this MGPS to higher cortisol secretion in response to laboratory threat, Pagliaccio et al., 2014); however, sustained hyperactivity of the HPA axis may ultimately result in a relatively flat diurnal pattern despite the continued presence of chronic stress. Thus, studies examining genetic moderation of the relationship between acute or daily stress exposure

on diurnal slope may reveal different patterns of results.

Previous research on multilocus HPA-axis genetic variation has focused on depression and related phenotypes (e.g., anxiety) as outcomes (Di Iorio et al., 2017; Feurer et al., 2017; Pagliaccio et al., 2014, 2015; Starr and Huang, in press). However, depression is one of many health conditions related to HPA-axis functioning. For example, flatter diurnal cortisol slope has been linked to fatigue, obesity, inflammation, cancer, externalizing problems, and mortality risk (see Adam et al., 2017 for a review). Our results suggest MGPS intensifies effects of stress on fatigue, which could be reflective of general health functioning. As such, HPA-axis multilocus genetic variation may be relevant to other health-related outcomes, as previous research found individual HPA-axis-related polymorphisms moderate associations between stress exposure and indices of physical health (e.g., Lessard and Holman, 2014; Suarez et al., 2017).

The current study is the first to our knowledge to investigate relationships between MGPS-defined genetic risk and diurnal cortisol patterns in an adolescent sample. Adolescence presents a critical developmental period to examine these processes, as increases in the intensity and complexity of novel stressors across multiple domains may challenge biological stress response systems in new, intensive, and repetitive ways. The circadian rhythm of cortisol secretion also exhibits an age-related developmental trajectory, as evidence suggests mean cortisol levels increase from childhood to middle adolescence and diurnal slopes flatten. However, this pattern is non-linear, as cortisol levels display a “U-shape” curve (Schreiber et al., 2006; Shirtcliff et al., 2012). In a study of adolescents aged 13–19, Adam (2006) found that diurnal curves increase with pubertal stage, although with steeper diurnal decline. It is possible that the interaction between genetic risk and chronic stress predicting diurnal cortisol patterns is especially salient during adolescence, with differential patterns in childhood or adulthood.

4.1. Study limitations

Some limitations should be considered. First, saliva collection was tracked using electronic compliance monitoring in only a subset of the sample, and we did not confirm wake-time using actigraphic methods. Recently published standards recommend objective assessment of sampling accuracy and adherence (Stalder et al., 2016), and although these standards are specific to CAR, sampling non-compliance can also affect diurnal slope, with studies using strict compliance monitoring yielding higher effect sizes (Adam et al., 2017). That said, sampling compliance for the current study is well within accepted parameters for daily ambulatory assessment (Kudielka et al., 2003). Second, we used only two time points across two days to assess diurnal slope (waking and waking + 12); although we collected two additional samples, these were timed to capture CAR, which is a separate phenomenon from diurnal slope (Wilhelm et al., 2007). Although two samples are often used to calculate diurnal slope, recent meta-analytic evidence suggests use of three or more samples is associated with higher effect sizes in predicting health outcomes (Adam et al., 2017). Further, use of three or more days of sampling (while not associated with increased effect sizes; Adam et al., 2017) would have permitted analysis of within-subject associations (e.g., between same- or previous-day stressors and slope). Although ambulatory cortisol collection presents inherent challenges with school-aged adolescents (Halpern et al., 2012), current results should be interpreted with appropriate caution and replication using more rigorous assessment methods is needed.

Additionally, fatigue and NA were collected on the same days that diurnal slope was calculated, so we are unable to draw inferences about temporal precedence. Indeed, previous research has supported a dynamic, transactional relationship between diurnal cortisol rhythms and emotional well-being (Adam, 2006; although alterations in slope appear to precede fatigue; Kumari et al., 2009). It is possible that the strength of these momentary processes is modified by genetic risk (e.g., NA more strongly predicts altered diurnal slope among genetically vulnerable adolescents). Likewise, it is possible that MGPS increases risk for NA/fatigue via other biological pathways, and that mood/fatigue in turn influence diurnal slope. Finally, we utilized a relatively small sample for a genetic study, as methodological standards have recently intensified for candidate gene research following prominent non-replications and concerns over low power (Dick et al., 2015). However, these more stringent standards were developed for single polymorphism candidate gene studies; multilocus genetic profiles substantially bolster statistical power and are likely appropriate for more moderately sized samples. Moreover, we used a pre-established MGPS (Pagliaccio et al., 2014) which, although promising, does not capture all sources of genetic variation in HPA axis activity. For example, recent GWAS analyses identified the SERPINA6/SERPINA1 locus (encoding corticosteroid binding globulin) as predictive of plasma cortisol (Bolton et al., 2014). Included loci are unevenly represented (five SNPs for CRHR1, three for NR3C1, and only one each for NR3C2 and FKBP5), and some SNPs selected for associations with depression and related phenotypes rather than HPA activity. Although refinements to this MGPS may eventually be needed, the current study adds to growing evidence that it captures genetic variation consequential for biopsychosocial functioning. These limitations are balanced by several study strengths, including use of an MGPS approach that captures the effect of additive genetic risk, an adolescent sample, and gold-standard interview methods to assess chronic stress.

4.2. Conclusions

Findings extend understanding of the interactive relationship between genetic risk and stress in contributing to neuroendocrine dysregulation and negative outcomes and highlight the importance of considering contextual factors to examine the complex interplay between genetic variation, environment, and physiological factors in the development of psychopathology. Our results highlight possible diatheses and mechanisms for the development of negative clinical and health outcomes following chronic stress, therefore potentially providing targets for future interventions.

Conflict of interest statement

The authors report no conflicts of interest.

Author statement

LS and KD collaboratively conceptualized present analyses. LS was the principal investigator for the overall project, conducted all formal analyses, and played the primary role in writing the original draft. KD provided methodological consultation and data cleaning assssistance. KD, YIL, and ZAS contributed to writing the original draft and to review and editing. YIL and ZAS contributed to project administration.

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