Cortisol awakening response and additive serotonergic genetic risk interactively predict depression in two samples: The 2019 Donald F. Klein Early Career Investigator Award Paper

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Abstract:

Background The serotonin system and hypothalamic pituitary-adrenal (HPA)-axis are each implicated in the pathway to depression; human and animal research support these systems' cross-talk. Our work implicates a 5-variant additive serotoninergic multilocus genetic profile score (MGPS) and separately the cortisol awakening response (CAR) in the prospective prediction of depression; other work has shown that the serotonin transporter polymorphism 5HTTLPR predicts CAR and interacts with the CAR to predict depression.
Methods We tested the hypothesis that a 6-variant MGPS (original plus 5HTTLPR) would interact with CAR to predict prospective depressive episode onsets in 201 emerging adults using four annual follow-up interviews. We also tested whether MGPS predicted CAR. We attempted replication of significant findings in a sample of 77 early adolescents predicting depression symptoms.
Results In sample 1, MGPS did not significantly predict CAR. MGPS interacted with CAR to predict depressive episodes; CAR slopes for depression steepened as MGPS increased, for risk or protection. No single variant accounted for results, though CAR's interactions with 5HTTLPR and the original MGPS were both significant. In sample 2, the 6-variant MGPS significantly interacted with CAR to predict depression symptoms.
Conclusions Higher serotonergic MGPS appears to sensitize individuals to CAR level—for better and worse—in predicting depression.

Keywords: cortisol awakening response | depression | emerging adults | multilocus | serotonin

Article:

Despite controversy around candidate gene research and specifically the serotonin transporter-linked polymorphic region 5HTTLPR (Culverhouse et al., 2018; Karg, Burmeister, Shedden, & Sen, 2011), prevailing theoretical models of genetic risk for depression indicate that the small effects of many genetic variants additively contribute to depression risk (Bogdan, Baranger, & Agrawal, 2018). We provided evidence across two samples that a such an additive genetic effect—a multilocus genetic profile score (MGPS)—comprising five serotonergic polymorphisms other than 5HTTLPR interacted with interpersonal stress to predict depression (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). This effect was recently replicated (Starr, Vrshek-Schallhorn, & Stroud, under review).

The hypothalamic–pituitary–adrenal (HPA) axis is also implicated in depression risk. For example, morning cortisol levels and the cortisol awakening response (CAR, the on-average large increase in cortisol that occurs upon awakening) have been shown to prospectively predict depression (Adam et al., 2010; Goodyer, Tamplin, Herbert, & Altham, 2000; Hardeveld et al., 2014; Stroud, Vrshek-Schallhorn, Norkett, & Doane, under review). We found that the CAR remained a significant predictor for 2.5 years in a sample followed for 4 years (Vrshek-Schallhorn et al., 2013). (Several studies that found nonsignificant results for CAR's prospective effect have aimed to predict depression or its symptoms either at a single point or over a timespan beyond which CAR would be expected to remain predictive; Carnegie et al., 2014; LeMoult, Ordaz, Kircanski, Singh, & Gotlib, 2015; Schuler et al., 2017.)

Further, human and animal studies evince that the serotonin (5HT) system has complex bidirectional effects with the HPA axis. Cortisol suppresses serotonergic tone through numerous pathways (Leitch, Ingram, Young, McQuade, & Gartside, 2003; Maes et al., 1990; Rubin, 1967; Tafet, Toister-Achituv, & Shinitzky, 2001). Serotonin can stimulate cortisol release via the 5HT-1A, -2A, and -2C receptors in several regions such as the paraventricular nucleus of the hypothalamus, the pituitary, and the adrenal gland, but can also inhibit its activity (Lowry, 2002), thereby suggesting the potential for a bidirectional feedback loop. Evidence of cross-talk between these systems aligns with theoretical conceptualizations of both systems as contributing to energy regulation, the serotonin system through modulation of behavioral inhibition/disinhibition (Spoont, 1992) and the HPA axis through its facilitation of resource mobilization (Vrshek-Schallhorn, Avery, Ditcheva, & Sapuram, 2018).

Serotonergic genetic variation provides a means of probing HPA and serotonin relationships. Indeed, in one investigation, carriers of the 5HTTLPR S-allele (the putatively riskier variant) had higher morning cortisol levels, and morning cortisol was significantly more predictive of prospective depression in S-carriers than L/L homozygotes (Goodyer, Bacon, Ban, Croudace, & Herbert, 2009). Interestingly, this interaction effect fit a differential susceptibility model—one in which a genetic variant confers sensitivity to context for better or worse, rather than simply elevated risk under poor conditions (Belsky & Pluess, 2009). In this case, prediction of depression by morning cortisol was significantly more potent for S-carriers than for L/L homozygotes, for whom the effect of morning cortisol was nonsignificant. Further consistent with differential susceptibility, for S-carriers, low morning cortisol was associated with relatively lower risk than L/L, while high morning cortisol was associated with higher risk than L/L.

The present work sought to replicate and extend these findings in a sample of late adolescents who were followed into young adulthood, and an independent replication sample of early adolescents followed prospectively for 1 year. We hypothesized that an augmented 6-variant

serotonergic MGPS consisting of our original 5-variant MGPS (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015) plus 5HTTLPR would (a) predict higher CAR, and (b) interact with CAR to prospectively predict depression, such that higher MGPS values would correspond to greater influence of the CAR for depression. Evidence for these predictions could be clinically important because it would suggest that some individuals are more sensitive that others to their own HPA axis functioning—and therefore possibly also more sensitive to pharmacological or psychosocial interventions that target this system.

1 PRIMARY SAMPLE MATERIALS AND METHOD

Participants

Prior reports detail the recruitment and demographics of the larger Youth Emotion Project (YEP) sample and its cortisol sampling subsample (Adam et al., 2010). Briefly, high school juniors were oversampled for high neuroticism from a screener; 627 consented to a longitudinal study in which they completed annual interviews and questionnaires. Two-thirds (491) were invited and 344 participants (250 females) completed a baseline cortisol assessment. Participants provided DNA (n = 410) during the follow-up period. Individuals excluded from analyses (for one or more reasons) took corticosteroid medications (n = 14); provided insufficient cortisol data (n = 10); were missing > 1 genotype call (n = 6); completed no follow-up interviews within 4 years of baseline (n = 15); had not completed baseline questionnaires (n = 24); were diagnosed with major depression at the baseline SCID or cortisol sampling (n = 13); were diagnosed with baseline PTSD (n = 1) associated with lower cortisol levels (for a meta-analysis see Meewisse, Reitsma, De Vries, Gersons, & Olff, 2007); or at any time during the study period were diagnosed with dysthymic disorder (n = 7), clinically significant psychotic symptoms (n = 3), or bipolar I/II (n = 5). These diagnoses were excluded a priori from analyses to provide a cleaner comparison group. In total, 201 participants remained (146 females), aged 17.1 years (SD = 0.4) at the CAR measurement, and self-identified as white (50.2%), African American (9.5%), Hispanic or Latino (15.4%), Asian (5%), or multiple/other races and ethnicities (19.9%).

Materials and procedures

1.2.1. Diagnostic interviews

A Structured Clinical Interview for DSM-IV, non-patient edition (SCID; First, Spitzer, Gibbon, & Williams, 2001) assessed lifetime diagnoses of mental disorders including major depressive episodes (MDEs) at baseline followed by four annual follow-up SCIDs assessing MDE diagnoses since the prior interview. Interviewers presented all cases to a doctoral supervisor and were blind to prior assessments. Interrater reliability for MDE diagnoses for ~10% of YEP SCIDs (across five interview waves, $\kappa = 0.82–0.94$; M = 0.89, SD = 0.05).

1.2.2. DNA collection, genotyping, and risk score construction

Participants provided salivary DNA samples via Oragene collection kits (DNA Genotek, Ontario, Canada) which were genotyped using KASP™ assays as previously described (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). All variants satisfied Hardy–Weinberg equilibrium (HWE)

$\chi 2s \leq 3.369$, $Ps \geq 0.066$. MGPS was constructed as previously reported (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015) except that we augmented the MGPS by including 5HTTLPR genotype, coding S as risky. Genotypes were coded for the number of risk alleles (0–2), except for (a) 5-HTTLPR which was coded 0 or 1 representing S-carriers, consistent with prior work in this sample (Vrshek-Schallhorn et al., 2014) and Goodyer's report (2009); and (b) HTR2C's rs6318, which is X-linked. This SNP was coded as 0/1 because males have one copy; prior work indicates that its influence over cortisol reactivity to laboratory-based stress did not vary by sex when coded this way (Avery & Vrshek-Schallhorn, 2016). Risk alleles were summed, permitting one missing genotype per person via prorating risk alleles; scores were centered for analyses.

1.2.3. CAR assessment

CAR was assessed as previously described (Adam et al., 2010; Doane et al., 2013). Briefly, on 3 consecutive weekdays, cortisol was assessed by passive drool, including immediately at waking, 40 min following waking and at bedtime. Participants completed questionnaires to assess covariates (asthma, hormonal contraceptives, time of waking and bedtime, nicotine use) and a diary before each sampling including self-reported mood. On return to the laboratory, samples were stored at −20°C then shipped to Trier, Germany, for duplicate assay (DELFIA; Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). Intra-assay variation was 4.0–6.7%; interassay variation was 7.1–9.0%. Cortisol data were ln-transformed due to positive skew. CAR was calculated as the difference between the waking sample and the sample 40 min later and represents the mean from 3 days. Values were standardized for analyses.

Analytic Approach

1.3.1 Regression

Multiple linear regressions tested Hypothesis 1, whether the risk score predicted CAR. A first model did not include covariates, and a second model included an array of recommended CAR covariates (gender, African American race, Latino/a ethnicity, waking time, nicotine use, hours of sleep, asthma, oral contraceptive use, waking cortisol level, age at CAR, episodic stress level in the 2 months before CAR) to ensure stable results (Stalder et al., 2016).

1.3.2 Survival analyses

Cox regression (continuous-time survival analysis) tested Hypothesis 2, whether the 6-variant risk score interacted with the CAR to predict MDE onset (herein called a gene-by-neuroendocrine [G×NE] effect). In models covarying gender, main effects of the CAR and the 6-variant MGPS were entered before their interaction effect, calculated using centered variables. Follow-up analyses to the primary G×NE model (a) addressed whether any significant interaction effects identified for the 6-variant risk score would persist: (b) when tested in the largest racially homogeneous subsample to rule out population stratification, (c) when partially deconstructed into interactions of CAR with each of 5HTTLPR and our original 5-variant score, (d) in an exploratory test of whether the effect varied by time, given that the CAR effect significantly declines over time, and (e) above and beyond any effect of neuroticism, and (f) above and beyond common CAR

covariates. Finally, following significant G×NE effects, to ascertain the direction of effect and estimated effect size of each variant, we planned to repeat the G×NE effects with each individual variant. Because individual variants each contribute small effects, significant effects for the individual variants were unexpected, save for 5HTTLPR.

Consistent with prior reports (Vrshek-Schallhorn et al., 2014; Vrshek-Schallhorn, Stroud, Mineka, Hammen, et al., 2015), we conducted person-month survival analyses. CAR data collection represented the beginning of the study period. Dates of MDE onset and offset were coded to the nearest month. Those with depression at the beginning of this period were excluded from analyses until recovery (e.g., Kendler, Kuhn, Vittum, Prescott, & Riley, 2005); all months following an MDE onset were excluded until recovery. MDE onsets were coded as absent versus present each month (0/1). There were 41 MDEs (first onsets and recurrences) in 8,190 person-months. Hazard ratio (HR) effect sizes represent the change in the likelihood of an MDE accompanying any one unit predictor increase (Singer & Willett, 2003).

1.3.3. Multiple testing

Two initial tests were conducted for Hypothesis 1, with and without covariates; false discovery rate correction was planned in the event of significant findings. One initial test was conducted with the MGPS for Hypothesis 2 (the G×NE effect); as follow-up tests of individual SNPs were planned only if the G×NE effect obtained in the single test was significant, corrections were not applied.

2 PRIMARY SAMPLE RESULTS

Table 1 presents zero-order correlations and descriptive statistics. Multiple linear regression did not support that the risk score predicted the CAR (Hypothesis 1), $B = -0.03$ standard error (SE) $(B) = 0.042$, $t = -0.706$, $P = 0.481$), and the post hoc addition of covariates did not change this outcome, $B = -0.030$, $SE(B) = 0.040$, $t = -0.740$, $P = 0.460$. Because results were nonsignificant, we neither proceeded to correct for multiple tests, nor attempted replication in the second sample.

Table 2 provides full model results for Hypothesis 2 and its follow-up tests. The primary model 2a (Table 2a) indicated that the CAR significantly interacted with the 6-variant serotonergic risk score ($HR = 1.485$, $P = 0.008$).1 Simple main effects of the slope of CAR at ± 1 SDs of the mean of MGPS indicated that the CAR had a nonsignificant negative slope at lower values of the MGPS ($HR = 0.820$, $P = 0.473$), but significantly and positively predicted MDE onset at higher values of the MGPS ($HR = 2.272$, $P = 0.002$) (Figure 1). This G×NE effect persisted in the white subsample ($HR = 1.772$, $P = 0.003$; Table 2b), ruling out population stratification; remaining analyses were conducted in the full sample. To replicate Goodyer et al. (2009), the 6-variant risk score was decomposed into 5HTTLPR and the original 5-variant risk score: both G×NE effects were significant: 5HTTLPR ($HR = 2.923$, $P = 0.014$) and the 5-variant MGPS ($HR = 1.389$, $P = 0.026$; Table 2c). As an additional post hoc test, substituting either the waking cortisol sample or the sample taken 40 min after waking for the CAR variable did not produce a significant interaction with the MGPS (Ps = 0.152 and 0.155, respectively; full models available on request).

Table 1. Primary sample descriptive statistics and zero order Pearson's correlations of central variables

	M	SD	1	2	3	4	5	6	7	8	9	10
1. Gender (Male = 1, Female = 0)	0.2736	0.44694										
2. Neuroticism composite score	−0.039	0.78714	−0.108									
3. 5HT1A G-alleles (0–2)	1.015	0.7534	−0.027	−0.017								
4. 5HTR2A C-alleles (0–2)	1.805	0.43349	0.071	−0.102	−0.191*							
5. 5HTR2C C-carrier (0 vs. 1)	0.2359	0.42565	−0.095	−0.065	−0.011	−0.049						
6. TPH2 rs11178997 T-alleles (0–2)	1.815	0.42624	0.136	−0.090	0.024	−0.089	−0.070					
7. TPH2 rs4570625 G-alleles (0–2)	1.445	0.6073	0.084	−0.100	−0.015	−0.087	−0.057	0.575*				
8. 5HTTLPR S-carrier (0–1)	0.6816	0.46702	−0.084	0.072	−0.029	−0.086	−0.081	0.059	0.008			
9. 6-variant 5HT profile score	7.009	1.29831	0.033	−0.115	0.504*	0.111	0.227*	0.585*	0.602*	0.316*		
10. Cortisol awakening response	0.2429	0.76987	−0.129	−0.020	0.122	−0.169*	0.015	−0.077	−0.103	0.001	−0.054	
11. No. of MDE onsets in study period	0.1493	0.62258	−0.148*	0.238*	0.038	0.072	−0.059	−0.151*	−0.084	0.044	−0.057	0.137

Note. MDEs reported in table as total number in study period, but analyzed in survival models on a person-month basis as occurring (1) or not (0) each month. Cortisol awakening response (μg/dl) is presented here unstandardized but was analyzed standardized for centering.
MDE: major depressive episode; M: mean; SD: standard deviation.
* Significant at 0.05 level.
** Significant at 0.01 level.

Table 2. Primary sample results for additive 5HT multilocus genetic profile score (MGPS) interacting with cortisol awakening response (CAR) to predict MDEs

				95% CI for HR		
	B	**SE(B)**	**HR**	**Lower CI**	**Upper CI**	**P value**
2a. Primary model: Full sample						
CAR	0.304	0.186	1.355	0.941	1.952	0.102
6-Variant 5-HT MGPS	−0.175	0.113	0.840	0.672	1.048	0.123
CAR × 6-Variant 5-HT MGPS	0.396	0.150	1.485	1.107	1.994	0.008
2b. Primary model repeated: White subsample						
CAR	−0.018	0.254	0.982	0.597	1.616	0.944
6-Variant 5-HT MGPS	−0.432	0.176	0.649	0.460	0.918	0.014
CAR × 6-Variant 5-HT MGPS	0.572	0.195	1.772	1.210	2.595	0.003
2c. Partially deconstructed risk score model						
S-Carrier 5-HTTLPR	−0.096	0.387	0.909	0.426	1.939	0.804
5-Variant 5HT MGPS (without 5-HTTLPR)	−0.190	0.120	0.827	0.654	1.046	0.113
CAR	−0.454	0.384	0.635	0.299	1.348	0.237
CAR × S-Carrier 5-HTTLPR	1.074	0.439	2.928	1.239	6.918	0.014
CAR × 5-Variant 5HT MGPS (without 5-HTTLPR)	0.328	0.148	1.389	1.039	1.856	0.026
2d. Accounting for CAR × Time effect						
CAR	0.776	0.272	2.173	1.275	3.704	0.004
6-Variant 5-HT MGPS	−0.246	0.184	0.782	0.545	1.121	0.180
CAR × Centered time	−0.031	0.014	0.970	0.944	0.996	0.026
6-Variant 5-HT MGPS × Centered time	0.005	0.010	1.005	0.985	1.024	0.652
CAR × 6-Variant 5-HT MGPS	0.412	0.152	1.509	1.121	2.032	0.007
2e. Prediction beyond neuroticism						
CAR	0.403	0.204	1.496	1.003	2.231	0.049
6-Variant 5-HT MGPS	−0.041	0.134	0.960	0.738	1.249	0.760
Neuroticism composite	1.619	0.259	5.046	3.039	8.379	<0.001
CAR × 6-Variant 5-HT MGPS	0.431	0.158	1.538	1.129	2.096	0.006
2f. Prediction beyond common CAR covariates						
Male	−0.883	0.565	0.413	0.137	1.251	0.118
Black race	0.536	0.558	1.709	0.573	5.099	0.336
Hispanic ethnicity	0.562	0.420	1.755	0.770	3.998	0.181
Waking time	−0.404	0.201	0.668	0.450	0.991	0.045
Hours of sleep	−0.378	0.185	0.685	0.477	0.984	0.040
Asthma	−0.095	0.642	0.909	0.258	3.200	0.882
Birth control (women)	−1.075	0.643	0.341	0.097	1.203	0.094
Negative emotion at CAR	1.494	0.542	4.455	1.541	12.876	0.006
Fatigue at CAR	0.614	0.453	1.847	0.760	4.492	0.176
Nicotine use	1.706	0.619	5.508	1.636	18.543	0.006
Age at CAR	0.294	0.460	1.342	0.545	3.305	0.523
Waking cortisol level	0.611	0.373	1.842	0.887	3.827	0.102
CAR	0.520	0.262	1.682	1.006	2.810	0.047
6-Variant 5-HT MGPS	−0.150	0.129	0.861	0.668	1.109	0.247
CAR × 6-Variant 5-HT MGPS	0.604	0.196	1.829	1.245	2.686	0.002

Note. Gender was covaried throughout but presented in the final model. Models represent the final step.
CI: confidence interval; HR: hazard ratio; MDE: major depressive episodes; SE: standard error.

Additional robustness tests examined the G×NE effect's stability. Given our prior work showing that CAR's effect significantly degrades with time (a CAR × Time interaction), we added the CAR × Time interaction to the model: the G×NE effect persisted (HR = 1.509, P = 0.007; Table 2d). The G×NE effect also persisted when neuroticism (HR = 1.538, P = 0.006; Table 2e) and common CAR covariates (HR = 1.829, P = 0.002; Table 2f) were added to the model.

Analyses examining the G×NE effect for individual variants with CAR (in separate models) found significant G×NE effects for 5HTTLPR; those for 5HT2A C-alleles (P = 0.073) and TPH2 rs4570625 G-allele (P = 0.090) approached significance (Table 3).

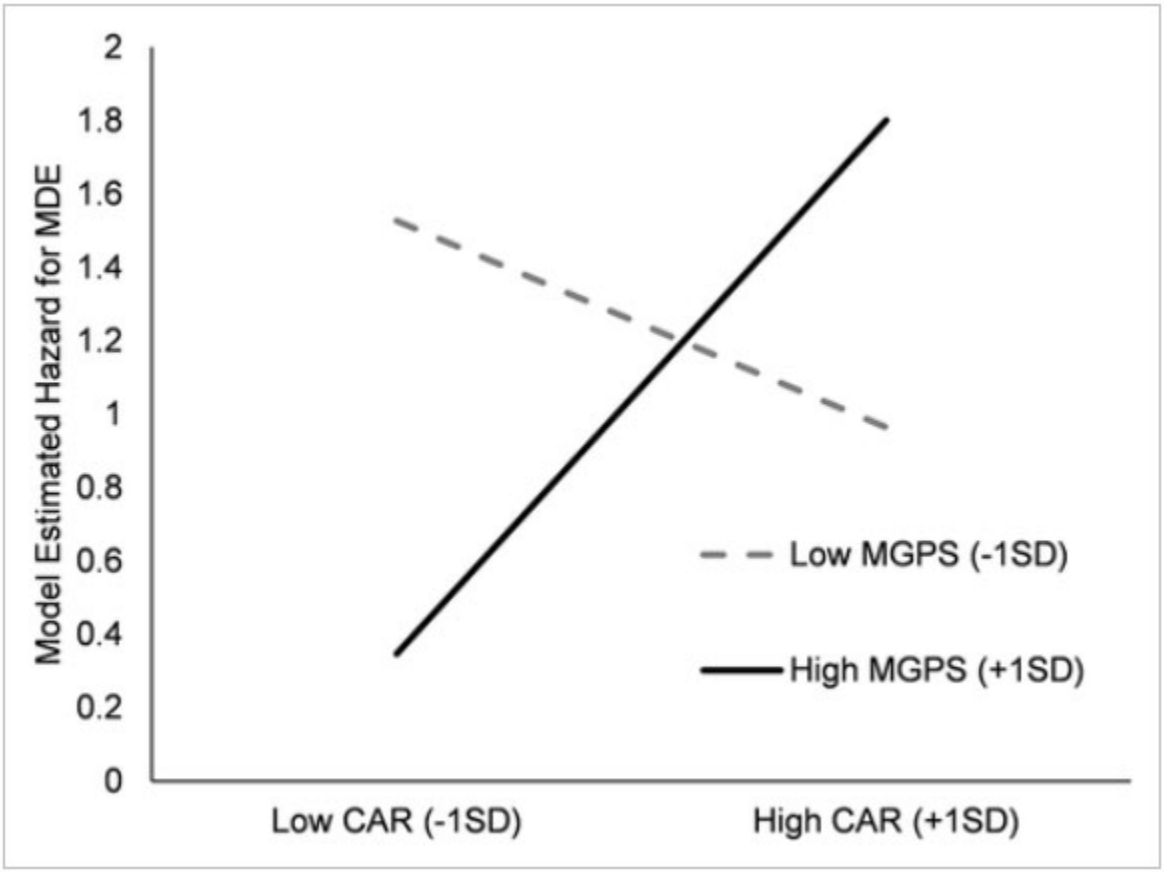

Figure 1. Primary sample model estimated hazard for major depressive episode onset as a function of CAR and MGPS. Solid black line represents high MGPS slope value of CAR, P = 0.002. Dotted gray line represents low MGPS slope value of CAR, P = 0.473. CAR: cortisol awakening response; MGPS: 6-variant serotonergic multilocus genetic profile score; SD: standard deviation

3 REPLICATION SAMPLE MATERIALS AND METHOD

Participants and overview

We sought to replicate key significant results of the primary sample in an independent sample drawn from a study investigating biopsychosocial predictors of psychopathology (full sample N = 126; Stroud, Chen, Doane, & Granger, 2018). At baseline (T1) and the 1-year follow-up

(T2), daughters completed interviews. Approximately 1 week after T1 (M = 7.48 days; SD = 8.86), adolescents collected whole saliva by passive drool at waking, 30 min post-waking, and bedtime, for 3 consecutive weekdays. For each sample, adolescents recorded the time and completed a diary. To obtain objective collection times, MEMS 6TM (AARDEX; Aardex Group, Richmond, VA) track caps were used. Of the 126 participants, 91 completed the collection and used the track cap. Participants who used the track cap (Stalder et al., 2016), who did not have a history of or current diagnosable depression, who provided a sufficient DNA sample, and who participated in T2 were included in analyses (N = 77; T1 M age = 12.33 years, SD = 0.72 years; 88.31% White).

Table 3. Primary sample post hoc individual variant interaction effects with cortisol awakening response (CAR) predicting MDEs

				95% CI for HR		
	B	**SE(B)**	**HR**	**Lower CI**	**Upper CI**	**P value**
CAR × S-Carrier	0.829	0.396	2.292	1.055	4.977	0.036
CAR × 5-HT1A G-Alleles	0.054	0.224	1.055	0.68	1.637	0.811
CAR × 5-HT2A C-Alleles	0.748	0.417	2.112	0.933	4.78	0.073
CAR × 5-HTR2C C-carrier	−0.325	0.416	0.722	0.32	1.632	0.434
CAR × TPH2 rs11178997 T-alleles	0.407	0.385	1.502	0.706	3.195	0.291
CAR × TPH2 rs4570625 G-alleles	0.511	0.302	1.667	0.923	3.01	0.090

Note. Main effects of CAR and genetic variants were included in models, but only interaction effects are presented to conserve space. Gender was covaried. Full models available on request.
CI: confidence interval; HR: hazard ratio; MDE: major depressive episodes; SE: standard error.

Materials and Procedures

3.2.1. Diagnostic Interviews

At T1 and T2, the Schedule for Affective Disorders and Schizophrenia for School-Aged Children-Present and Lifetime version (Kaufman et al., 1997) assessed current and lifetime depressive symptoms. Symptoms were rated: 0 = none; 1 = mild; 2 = moderate; 3 = DSM-IV criteria. T1 ratings reflect lifetime history and current symptoms. For T2 depressive symptoms, the worst period of symptoms between T1 and T2 served as the dependent variable (e.g., Vrshek-Schallhorn, Stroud, Mineka, Hammen, et al., 2015). Symptom level was predicted because full diagnoses are rare at this age. Inter-rater reliability: ICCs = 0.97–1.00.

3.2.2. DNA collection, genotyping, and risk score construction

At T1, adolescents provided a DNA sample using an Oragene collection kit (see Vrshek-Schallhorn, Stroud, Mineka, Hammen, et al., 2015 for full details). The 6-Variant 5-HT MGPS was calculated as in the primary sample. Single nucleotide polymorphisms (SNPs) satisfied HWE, $\chi 2s \leq 1.687$, $Ps \geq 0.194$.

3.2.3. Cortisol assessment

Samples were assayed in duplicate, using a solid phase time-resolved fluorescence immunoassay with fluorometric endpoint detection (DELFIA; Dressendörfer et al., 1992). The intra-assay coefficients of variation were 4.0–6.7%, and the interassay coefficients of variation were 7.1–9.0%. Consistent with other publications in this sample (Sladek, Doane, & Stroud, 2017; Stroud et al., under review), the CAR was calculated using the formula for area under the curve with respect to increase after natural log transformation to address skew. The mean CAR from all days available was used.

4 REPLICATION SAMPLE RESULTS

See Table 4 for zero-order correlations and descriptive statistics, and Table 5 for results. Hierarchical linear regression analyses predicting T2 depressive symptoms were used. The main effects of the CAR and the 6-variant serotonergic risk score were entered in Step 1 and their interaction in Step 2. All variables were standardized. The CAR significantly interacted with the 6-variant serotonergic risk score (Table 5a; Hypothesis 2a), such that CAR was more strongly predictive of prospective depressive symptoms (b = 1.1015; SE = 0.382; B = .548; P = 0.010) at high (1 SD above the mean) versus low (1 SD below the mean; b = 0.369; SE = 0.206; B = 0.199; P = 0.078) levels of the MGPS. This interaction persisted in the white subsample (Table 5b; Hypothesis 2b) and when adding CAR covariates (Table 5c; Hypothesis 2 f). As in the primary sample, substituting the waking sample or 30-min post waking sample for CAR did not produce significant interactions with the MGPS (Ps = 0.258, 0.805; full models available on request).

5 DISCUSSION

In two samples, we provide the first evidence that an augmented 6-variant additive serotonergic genetic profile score interacted with CAR levels to predict depression onset and symptoms. Further, although we did not extend a finding that 5HTTLPR predicts greater CAR levels (Goodyer et al., 2009) using the 6-variant profile score, using the CAR, we conceptually replicated and extended their finding that morning cortisol levels interact with 5HTTLPR. In our primary sample, both 5HTTLPR and the 5-variant serotonergic profile score each interacted with CAR to predict MDE onsets. The clinical implication of these findings is that some individuals (those with higher serotonergic MGPS scores) may be more sensitive to their own HPA-axis functioning—but also may benefit more from pharmacological interventions targeting this system.

Results illustrate a relatively novel manifestation of differential susceptibility theory—the idea that genes do not encode purely risk for negative outcomes, but instead encode sensitivity to aspects of the natural environment, leading to enhanced beneficial effects of better conditions but also worsened costs of poorer conditions (Belsky & Pluess, 2009). In this case, in place of the natural environment is the CAR, a G×NE effect. Importantly, the current findings replicate those identified by Goodyer et al. (2009) in which 5HTTLPR S-carriers were more sensitive to their morning cortisol levels for depression risk, as compared to their L/L counterparts (for whom CAR was not a significant predictor as a simple main effect). In both present samples, the slope for CAR was significantly more predictive of

Table 4. Replication sample: descriptive statistics and zero order Pearson's correlations of central variables

	M	SD	1	2	3	4	5	6	7	8
1. 5HT1A G-alleles (0–2)	1.156	0.650								
2. 5HTR2A C-alleles (0–2)	1.831	0.377	−0.052							
3. 5HTR2C C-carrier (0 vs. 1)	0.263	0.443	−0131	0.113						
4. TPH2 rs11178997 T-alleles (0–2)	1.777	0.456	−0.142	0.074	−0.017					
5. TPH2 rs4570625 G-alleles (0–2)	1.442	0.659	−0.255*	0.039	0.060	0.567**				
6. 5HTTLPR S-carrier (0–1)	0.757	0.432	−0.258*	−0.096	0.066	0.043	0.133			
7. 6-variant 5HT Profile Score	7.252	1.345	0.267*	0.319**	0.456**	0.591**	0.646**	0.276*		
8. Cortisol awakening response	0.077	0.198	−0.089	−0.073	0.058	−0.035	−0.078	−0.128	−0.035	
9. Time 2 depressive symptoms	0299	0.727	−0.095	−0.006	0.091	0.015	−0.114	−0.103	−0.014	0.123

Note. Cortisol awakening response = calculated as area under the curve with respect to increase (AUCi; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) with natural log transformation to address skew. N = 77. Note. M: mean; SD: standard deviation; SE: standard error.
* Significant at 0.05 level.
** Significant at 0.01 level.

Table 5. Replication sample: Hierarchical linear regression analyses examining the CAR, a 6-variant 5-HT MGPS, and their interaction in predicting depressive symptoms

	b	*SE*	*B*	*P*
5a. Primary model: Full sample				
CAR	0.369	0.206	0.199	0.078
6-Variant 5-HT MGPS	0.159	0.199	0.090	0.425
CARCAR × 6-Variant 5-HT MGPS	0.641	0.303	0.239	0.038
5b. Primary model repeated: White subsample				
CAR	0.383	0.199	0.224	0.058
6-Variant 5-HT MGPS	0.143	0.200	0.084	0.478
CARCAR × 6-Variant 5-HT MGPS	0.724	0.288	0.298	0.014
5c. Prediction beyond common CAR covariates				
Waking time	0.053	0.206	0.030	0.796
Mean daily negative affect	0.378	0.219	0.204	0.090
Mean daily positive affect	0.186	0.206	0.106	0.371
Age at CAR	−0.059	0.205	−0.032	0.775
Mean daily CAR noncompliance	0.449	0.193	0.254	0.023
CAR	0.343	0.210	0.184	0.107
6-Variant 5-HT MGPS	0.052	0.205	0.030	0.799
CAR × 6-Variant 5-HT MGPS	0.638	0.314	0.236	0.046

Note. All variables (except Time 2 depressive symptoms) were standardized. Cortisol awakening response = calculated as area under the curve with respect to increase (AUCi; Pruessner et al., 2003) with natural log transformation to address skew. Full sample n = 77. White sample n = 68. For 5c, n = 76 due to missing data on the covariates. Nicotine use and birth control pills were not included as covariates due to low frequency (e.g., Stroud et al., 2018). Models 5a and 5b were repeated with CAR computed as the difference between the waking sample and the sample 30 min later (log-transformed), and CAR × 6-Variant 5-HT Risk Score approached significance in Model 5a (b = 0.490; SE = 0.262; B = 0.210; P = 0.066) and was significant in Model 5b (b = 0.583; SE = 0.274; B = 0.274; P = 0.024).

These results may also inform inferences about the functioning of the serotonin system and cortisol in depression. Importantly, several of the "risk" alleles in the 6-variant profile score are associated with greater serotonergic tone, rather than lower tone as was originally implicated in monoamine theories of depression. The 5HTTLPR S-allele is associated with reduced transcription of the serotonin transporter, which recovers serotonin from the synaptic cleft back to presynaptic neurons (Lesch et al., 1996); lower transporter availability ought to lead to greater synaptic serotonin levels. Additionally, the C-allele of 5HT2C rs6318 confers greater receptor activity levels (Okada et al., 2004), which could behave similarly to an elevation in serotonin level. Moreover, the "riskier" T-allele of TPH2 SNP rs11178997 has been shown to confer greater transcriptional activity of TPH2 due to enhanced binding of an important transcription factor (Scheuch et al., 2007), which ought to lead to heightened serotonin production with increasing T-alleles. Although counter to the original monoamine hypothesis, these three observations mesh with more recent accounts that depression is associated with elevated serotonin turnover (production and metabolism; Barton et al., 2008) and potentially higher serotonergic tone (Andrews, Bharwani, Lee, Fox, & Thomson, 2015). Furthermore, these results suggest that there are distinct neurobiological pathways to depression as a function of MGPS—one in which the

HPA-axis is highly involved both for risk and protection (in those with high MGPS) and one in which it is not particularly critical (in those with low MGPS).

Finally, results illustrate the value of the MGPS approach for probing the influence and interrelationships of neurochemical systems. The ability to aggregate individually small effects from individual variants enhances the overall effect size versus typical single genetic variants (Starr & Huang,). Similarly, the dimensional nature of the MGPS variable enhances power over dichotomous variables (MacCallum, Zhang, Preacher, & Rucker, 2002). Further, this report suggests that 5HTTLPR can be reasonably added to the original 5-variant MGPS; however, as we have done here, to ensure that both it and the MGPS contribute to effects, it will be important to report findings with and without 5HTTLPR.

Limitations

Despite strengths including use of prospective measurement, clinical interview measures for depression, and a two-sample strategy, this report has limitations. Objective verification of wake-times (e.g., actigraphy) was not used for the CAR collection (Stalder et al., 2016). Additionally, the primary sample enriched for high levels of neuroticism to increase observed disorder onsets and may be nonrepresentative; a simulation study indicates this aids model convergence and does not bias results (Hauner, Zinbarg, & Revelle, 2014), and the effects presented here predicted above and beyond neuroticism (Table 2e).

6 CONCLUSION

Using a multilocus genetic profile score to capture serotonergic genetic variation, we provided novel evidence that additive serotonergic genetic variation interacts with the cortisol awakening response to predict major depressive episode onset in emerging adults and depressive symptoms in a replication sample of early adolescents. In both samples, higher values of the genetic profile score were associated with greater influence of the CAR in predicting depression, and nonsignificant effects of the cortisol awakening response were observed for lower genetic profile scores. Results suggest different pathways to depression for those with low serotonergic genetic risk, for whom the cortisol awakening response was not particularly important for predicting depression, versus for those with higher serotonergic genetic risk, for whom the cortisol awakening response was indeed predictive—both for better and for worse.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

Footnotes

1 We previously showed that the 5-variant serotonergic MGPS interacts with recent (past 2 months) major interpersonal stressful life events to predict MDE onsets in 387 YEP participants (HR = 1.815, P = 0.007; Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). In the cortisol subset analyzed here, the interaction of the 6-variant MGPS with major interpersonal stress persists (HR = 2.042, P = 0.020). In a model simultaneously examining both the G×NE effects reported here (i.e., CAR × MGPS) and the MGPS interaction with recent major interpersonal stressful life events, the MGPS interaction with CAR persisted (HR = 1.344, P = 0.048), while the interaction with major interpersonal stressful life events retained a similar effect size but approached significance, despite the substantial reduction in sample size (HR = 1.706, P = 0.084); full models are available from S. V.-S. Tests of asymptotic confidence intervals have not been extended to time-varying Cox models, thus testing mediation is premature, but neither effect appears to fully account for the other.

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